AQUATIC TERRITORY DEFENCE BY MALE HARBOUR SEALS (Phoca vitulina) AT MIQUELON: RELATIONSHIP BETWEEN ACTIVE DEFENCE AND MALE REPRODUCTIVE SUCCESS

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BY

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ABSTRACT

Pinnipeds have unique phylogenetic and environmental constraints which increase the potential for male polygyny. Most of the land-breeding species are polygynous. Less is known about the mating systems of water-breeding pinnipeds, like harbour seals. Harbour seals have many characteristics which suggest at least a low level of polygyny. The purpose of this study is to determine: 1) if male harbour seals are competing for females by displays and/or territorial maintenance and 2) whether these competitive tactics are linked to siring progeny.

The paternity results from DNA fingerprinting, using Jeffreys' 33.15 and 33.6 probes on a captive group, indicated that this technique could be used successfully to determine paternities in harbour seals. Observed copulation did not predict male reproductive success.

Paternity tests were conducted on five adult males and thirteen mother-pup pairs, caught in one study area at Miquelon. Two mother-pups pairs were excluded from the paternity analyses, as they had very low band-sharing coefficients suggesting that these females were fostering pups. Three of the displaying males had fathered pups, while one displaying male and non-displaying male had not.

The aquatic display behaviour, haul-out patterns and aggressive interactions of nine identified males were video-taped during two consecutive breeding seasons. Site-specific simultaneous displays occurred regularly between neighbouring males, establishing territory boundaries. Males defending more boundaries displayed at significantly higher rates than males with fewer boundaries. These results indicated that displays were for boundary defence and not self-advertisement. Intruder males were forced from haul-out areas through aggressive interactions. Territorial males did father some pups suggesting that there is reproductive success associated with territory defence. Some males were never seen displaying and may have been adopting an alternate mating strategy. The deep channels at Miquelon create physically bounded-water ways through which females and pups must pass. The topography at Miquelon appears to facilitate aquatic territory establishment and the defence of areas proximate to females.

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CHAPTER 1

MATING SYSTEMS

Little is known about the mating system of harbour seals (*Phoca vitulina*). It is difficult to determine their mating system because they are extremely wary (Renouf *et al.* 1981), making them difficult to observe, and they copulate in the water (Allen 1985), which makes it almost impossible to determine which males are acquiring successful copulations. Harbour seal females gather into predictable aggregations on beaches and rocky ledges for pupping and lactation (Lawson and Renouf 1985), after which they become sexually receptive (Bigg and Fisher 1975).

The clumping of females during the breeding season could make it possible for males to defend either the females themselves or a resource essential to the females (Emlen and Oring 1977). However, female harbour seals are somewhat synchronous in their oestrus. Based on pupping data from Miquelon, 95% of females become receptive within a 15 day period (Rosen 1990). This degree of synchrony would limit the number of females with which a male would be able to mate in a breeding season.

Evidence of fresh wounds and scars on male harbour seals during the mating period suggest that there is some degree of inter-male competition (Thompson 1988). In addition, there is a slight sexual dimorphism in harbour

seals with males being approximately six percent longer than females (McLaren 1993). All of these observations have lead researchers to speculate that harbour seals are slightly polygynous, such that one male probably mates with more than one female. The objectives of this study are to use DNA fingerprinting to determine the degree of polygyny of harbour seals at Miquelon, and to determine the behavioural forms of intermale competition that could result in the differential reproductive success among males.

To determine the mating system of a population, we need information on sex differences in variability of reproductive success, including information on how many mates are acquired, the type of pair bonds formed, and the extent to which both parents provide parental care (Emlen and Oring 1977). Theories from behavioural ecology on the evolution of mating systems strive to explain how individuals might increase their fitness, through inter- and intrasexual competition (eg. Orians 1969, Pianka 1976, Emlen and Oring 1977, Bradbury 1981, Oring 1982, Vehrencamp and Bradbury 1984, Clutton-Brock 1989, Boness 1991, Davies 1991).

Reproductive effort, the total amount of resources (time and energy) dedicated to reproduction, is often partitioned differently between the sexes. In general, females put more reproductive effort into parental care while males tend to expend more energy in mating effort (Trivers 1972). This is most apparent in polygynous species in which males expend a majority of their

reproductive effort defending territories or females, and have little involvement in caring for their young. This differential partitioning of reproductive effort arises because females produce fewer, more energetically costly gametes (Parker, Baker and Smith 1972, Alexander and Borgia 1978). In the case of mammals, gestation and lactation increase the energy expenditure per offspring for females compared to males. As a result of these mammalian characteristics, females are more likely to increase their reproductive success through successfully rearing their offspring, than to expend energy searching for new mates. Generally, females are expected to be more selective than males in choosing mates, as failure to choose the best mate would be more costly for them (Orians 1969, Trivers 1972). Conversely, males are capable of producing many more offspring per breeding season than females, and are only limited by the number of females with which they can mate.

Females may be able to increase their reproductive success by mating with superior males who will contribute to the future viability of their offspring. Females could assess male quality through parental care abilities (ex. female fish may test abilities of males to care for eggs, Kraak and Van Den Berghe 1992) or, in those cases where males do not invest in parental care, through contribution of high quality genes (Emlen and Oring 1977). To assess the quality of male genes, females could rely on physical attributes of males (Zahavi 1975, Hamilton and Zuk 1982), or outcomes of male-male competitions (Cox

and Le Boeuf 1977, Payne 1984, Watson 1990, Davies 1991). Female assessment may place males under additional selective pressure to succeed in intrasexual competition, which could explain the costly aggressive male-male encounters of some species (Riechert 1988; *e.g.* Clutton-Brock *et al.* 1982, Hogg 1987, Deutsch *et al.* 1990, Watson 1990). Therefore, males are expected to compete for as many females as possible to maximize their fitness, while females are expected to increase their fitness by mating with the "best" mates and ensuring survival of their young (Trivers 1972).

The extent to which intra- and intersexual competition can have a role in the evolution of mating systems is dependent on environmental factors that affect the temporal and spatial distribution of the sexes and resources. These distributions, in turn, influence the ability of one sex to monopolize, or control, resources or members of the other sex (Emlen and Oring 1977, Davies 1991). When neither resources nor individuals are defendable, or both parents must care for the young, then the mating system is expected to be monogamous (Davies 1991), which is defined here as mating exclusivity in a breeding season. The need for both parents to care for the young seems to be the most important factor in predicting monogamy in many seabirds, as the death or removal of one parent during incubation or chick rearing can result in complete breeding failure (Oring 1982). This, in part, may explain why monogamy is more common in birds than mammals; fewer than five percent of mammals are

suspected of being monogamous (Kleiman 1977, Dunbar 1984). In most cases of monogamy, males invest in their young through participating directly in parental care. In mammals, parental care usually involves activities such as providing food for lactating females, defending the young against predators and maintaining warmth in the natal nest (Alcock 1979, Wolff and Lidicker 1981).

It has been argued that internal gestation and lactation have freed most mammalian males from parental care duties thereby allowing them to take advantage of any environmental potential for polygamy (Orians 1969). It is also possible to argue that lactation, internal fertilization and gestation have reduced the ability of males to increase their reproductive success through investing effort in parental care. Therefore, increased reproductive success of most male mammals is tied to increased mating effort, the ability to attract and fertilize as many females as possible, while reducing the ability of other males to fertilize females (Low 1978).

When environmental conditions are such that mates or essential resources are defendable, then the potential for polygamy exists (Emlen and Oring 1977). Whether or not resources or individuals are energetically defendable will be influenced by their distributions in time and space. In general, if resources or individuals are clumped, or non-randomly distributed (Brown and Orians 1970), in both space and time, then there is a greater probability that they can be defended and that a polygamous mating system

will exist. A polygamous mating system is one in which a member of one sex mates with multiple members of the opposite sex. Polygyny describes the condition when one male mates with many females (it is assumed, sometimes erroneously, that the females mate with only one male). Polyandry describes the reverse. Polyandry, which occurs primarily in birds (Jenni 1974), is considered the rarest mating system and, therefore, will not be discussed here.

There are four forms of polygyny: female defence polygyny, resource defence polygyny, lek polygyny and scramble competition (Bradbury and Vehrencamp 1977, Emlen and Oring 1977, Schwagmeyer and Woontner 1986, Clutton-Brock 1989, Boness 1991). When females cluster in a predictable location at a predictable time, males may be capable of monopolizing them, and the mating system is likely to be female defence polygyny. In resource defence polygyny, resources essential to females are clumped and, therefore, males may be able to establish territories containing these resources, and thereby exert indirect control over the females. It is often difficult to distinguish between female defence and resource defence polygyny as females usually cluster in areas with abundant food, nesting sites and/or few predators (Emlen and Oring 1977, Boness 1991). The most obvious difference between the two is that males will move with the females they are defending in a female defence system while in a resource defence system males will not move from the

resource being defended, despite movement of females to and from the location.

Leks and scramble competition are relatively rare mating systems in mammals (Davies 1991). They tend to occur when environmental conditions are such that neither females nor resources are economically defendable (Emlen and Oring 1977, Bradbury 1981, Bradbury and Gibson 1983, Schwagmeyer and Woontner 1986). Lekking systems vary within and between species (Clutton-Brock *et al.* 1988, Pruett-Jones 1988, Apollonio *et al.* 1992) but, in general, males gather into dense aggregations in specific locations and defend small mating territories within these aggregations (Emlen 1976, Clutton-Brock *et al.* 1988). The territories contain no resources and males expend large amounts of energy in self-advertisement through visual, auditory or olfactory displays (Vehrencamp *et al.* 1989, Davies 1991). Females visit the leks, often visiting several males before copulating with one, making it possible for females to assess male quality based on the displays or the outcome of intra-sexual competition (Emlen and Oring 1977, Payne 1984, Kirkpatrick and Ryan 1991, Gibson *et al.* 1991). Territories differ in their quality (usually location within an arena), as measured by the relative number of matings occurring in each, and males compete for the better located territories (Emlen 1976, Apollonio *et al.* 1989a, 1989b, 1990, Festa-Bianchet *et al.* 1990). Presumably, higher quality males generally hold better territories, making it difficult to assess whether

males are selected by females on the basis of their displays or the attributes of their territories. It is often difficult to distinguish between defence of a territory on a lek and resource defence polygyny because there can be benefits to females associated with particular lek territories. For example, female Uganda kob (*Kobus kob thomasi*) prefer lek territories which contain little grass and are situated farther from thickets (in which predators can hide), thus reducing the threat of predation (Deutsch and Weeks 1992). However, female preference did not change when the territories were modified by reducing grass height or removing thickets, suggesting that females have a preference for particular lek territories (Balmford, Albon and Blakeman 1992).

The degree of sexual dimorphism within a species has been used as an indicator of the intensity of polygyny, particularly when males are substantially larger than females. Because size has an influence in males' abilities to obtain and defend either good quality territories or large harems (*e.g.* Le Boeuf 1974, Howard 1984), it is possible that larger male size is under some selective pressure. However, this is only likely if larger males also obtain significantly more successful copulations than smaller males. Deutsch *et al.* (1990) found that larger adult male northern elephant seals (*Mirounga angustirostris*) accounted for most of the copulations (only one subadult out of 29 managed to copulate). Unfortunately it is difficult to distinguish between the effects of size, age, and experience on the dominance rank and reproductive success of

males. Older, larger northern elephant seal males are usually the alpha males and account for a majority of the copulations (Le Boeuf 1974).

In scramble competition, males search or patrol for females and the likelihood of acquiring a mate is primarily dependent on mate-searching abilities and encounter rate (Schwagmeyer and Woontner 1985, 1986, Dickinson 1992). Scramble competition has been most frequently found in insects (Thornhill and Alcock 1983), but it also occurs in some anurans (Wells 1977), and has been documented in one species of mammal (Schwagmeyer and Woontner 1986). This mating system appears to arise under two environmental conditions: when females are widely dispersed and have slightly asynchronous oestrus, and when females are clustered in space, oestrus is synchronous and there is a high degree of male competition (Thornhill and Alcock 1983, Schwagmeyer and Woontner 1986). It is possible that the clumping of females and synchrony of oestrus has arisen to encourage male-male competition, thus allowing females to assess males and choose the best mates from the most successful males.

A major difficulty in trying to describe mating systems using the current vocabulary is that many of the systems have been described without a complete knowledge of the behaviour of both sexes. Too often, the mating system of a species is defined by the behaviour of the most dramatic sex while the behaviour of the remaining sex is assumed. As more individuals in

monogamous species (*e.g.* Quinn *et al.* 1989) and females in polygynous species (*e.g.* Gibbs *et al.* 1990) are found to be engaging in extra-pair copulations, there is a need for terms that do not define mating systems in terms of mating exclusivity of either or both sexes.

Not all mating systems fit into discrete categories but rather vary along a continuum with environmental conditions (Emlen and Oring 1977, Bradbury 1981, Boness 1991, Davies 1991, Le Boeuf 1991). As abundance of food or availability of breeding habitat changes, dispersion of females is likely to change and this will affect the form of mating system adopted by males (Bradbury and Vehrencamp 1977, Bradbury 1981, Davies 1991). There are many intraspecific examples of variations in mating strategies which appear to increase reproductive success of different behaviours under different environmental conditions (*e.g.* Alcock *et al.* 1977, Gibson and Bradbury 1987, Clutton-Brock *et al.* 1988, Pruett-Jones 1988, Hatchwell and Davies 1992a, 1992b).

Not all males in polygynous species compete equally well: dominant males are often larger and usually compete more successfully than smaller males (Le Boeuf 1974, McCann 1981, Sherman and Morton 1984, Le Boeuf and Reiter 1988, Deutsch *et al.* 1990, Godsell 1991, Balmford *et al.* 1992). For example, the highest ranked male southern elephant seal (*Mirounga leonina*)

accounted for almost 40 percent of the copulations by identified males (McCann 1981).

Subordinate males will often adopt alternate strategies in order to acquire matings (c.f Emlen 1976, Howard 1984, Arak 1988, Beehler and Foster 1988, Convey 1989, Gross 1991), but they may not be as successful as dominant males. For example, smaller male bullfrogs (*Rana catesbiana*) are not able to defend female-preferred territories as well as larger males and adopt an alternate strategy in which they intercept females approaching territorial males. These smaller satellite males accounted for less than three percent of the matings (Howard 1978, 1984). In many salmonid species, males mature at different ages and, while older males compete for mates, younger males sneak matings (Gross 1984). The success of the younger males is dependent on the density of sneaky males (Hutchings and Myers 1988).

Male bluegill sunfish adopt different mating strategies that appear to relate to age at maturity (Gross 1982). Seven to eight year old males employ a "parental" behaviour in which they construct nests in colonies, court schooling females, and provide parental care for the brood of fertilized eggs. Younger "cuckolder" males adopt two strategies, dependent on age and hence size. Smaller males, approximately two years of age, sneak into nests and spawn as females deposit eggs, while larger, four or five year old, males act as satellites and mimic female behaviour at the nest sites. In one study, Gross

(1982) determined that culkolders attempted to intrude into nests during approximately 60% of female dips (egg releases) but were only successful in spawning in 14% of these attempts. As the density of cuckolders increases, competition between them also increases to more than that between themselves and parental males, thus decreasing their spawning success (Gross 1991). Gross (1985) calculated that the lifetime fitness of male coho salmon employing two alternative mating strategies was approximately equal, despite being negatively frequency-dependent. Unfortunately, there are few studies in which fitness of males employing different strategies, has been assessed as it has been difficult to determine paternities in free-ranging animal populations (Burke 1989), particularly in polygynous species (Pemberton *et al.* 1992).

Many animal studies have relied on breeding behaviour and copulations as indications of mating strategies and measures of reproductive success (*e.g.* Le Boeuf 1974, Howard 1979, Clutton-Brock *et al.* 1982, Anderson and Fedak 1985). However, factors such as sperm competition (competition between sperm from several donors to fertilize an egg) and "sneaky" strategies underscore the need for better measures of paternity and, hence, male reproductive success. For example, a study of red-winged blackbirds (*Agelaius phoeniceus*) dispelled the assumption that females only mate with the male in whose territory their nests are located (Gibbs *et al.* 1990). Another study on shags (*Phalacrocorax aristotelis*) demonstrated that females will approach and

copulate with males other than the one with whom they incubate and raise their young (Graves *et al.* 1992). Therefore, shag males can have increased reproductive success through extra-pair copulations but decreased reproductive success from caring for young sired by another male.

The lesser snow goose (*Anser caerulescens*) provides another example of a species in which sneaky strategies are employed. This species is considered monogamous, pairing for life. Field observations have found that extrapair copulations (EPC) occasionally occur (Cooke and Rockwell 1988) and that females will occasionally dump eggs in nests other than their own (intraspecific brood parasitism, IBP) (Cooke and Mirsky 1972). These "sneaky" behaviours make it difficult to assign parentage. Fortunately, there are two colour morphs in this species which can be helpful in determining paternity and, therefore, assessing male fitness. More recently, species-specific DNA probes have been developed for DNA fingerprinting (Quinn and White 1987). These probes have been used to answer the specific genetic relatedness questions by making it possible to assign paternity and maternity in single broods (Quinn *et al.* 1987, Quinn *et al.* 1989). With the development of molecular techniques, such as DNA fingerprinting, it is now possible to assess male fitness within mating systems (Burke 1989; Boness, Bowen and Francis 1993), as will be attempted in this study.

PINNIPED MATING SYSTEMS

As in most other mammals, female pinnipeds (seals, sea lions and walruses) provide the complete nutritional requirements of their offspring. This frees males from parental care duties, and increases the potential for polygyny. In addition, pinnipeds are thought to have unique phylogenetic and environmental constraints which further increase this potential (see Figure 1.1). Bartholomew (1970) developed a model for the evolution of pinniped polygyny contingent on a combination of two features which separate pinnipeds from all other mammals: terrestrial parturition and aquatic feeding. He pointed out that pinnipeds have special physiological and anatomical adaptations for foraging in the aquatic medium, some of which present constraints (e.g. restricted terrestrial mobility), and live an amphibious lifestyle except during the breeding season. For the breeding season, females gather to give birth to and care for their young on land or ice which results in predictable aggregations of females in time and space, and thus increases the potential for polygyny. All of the pinniped species which copulate on land are clearly polygynous (Bartholomew 1970, Stirling 1975a, 1983, Boness 1991, Le Boeuf 1991), however, the mating systems of those species which gather on land or ice for pupping, but mate in water are not as well understood and appear to be more variable (see Table 1.1).

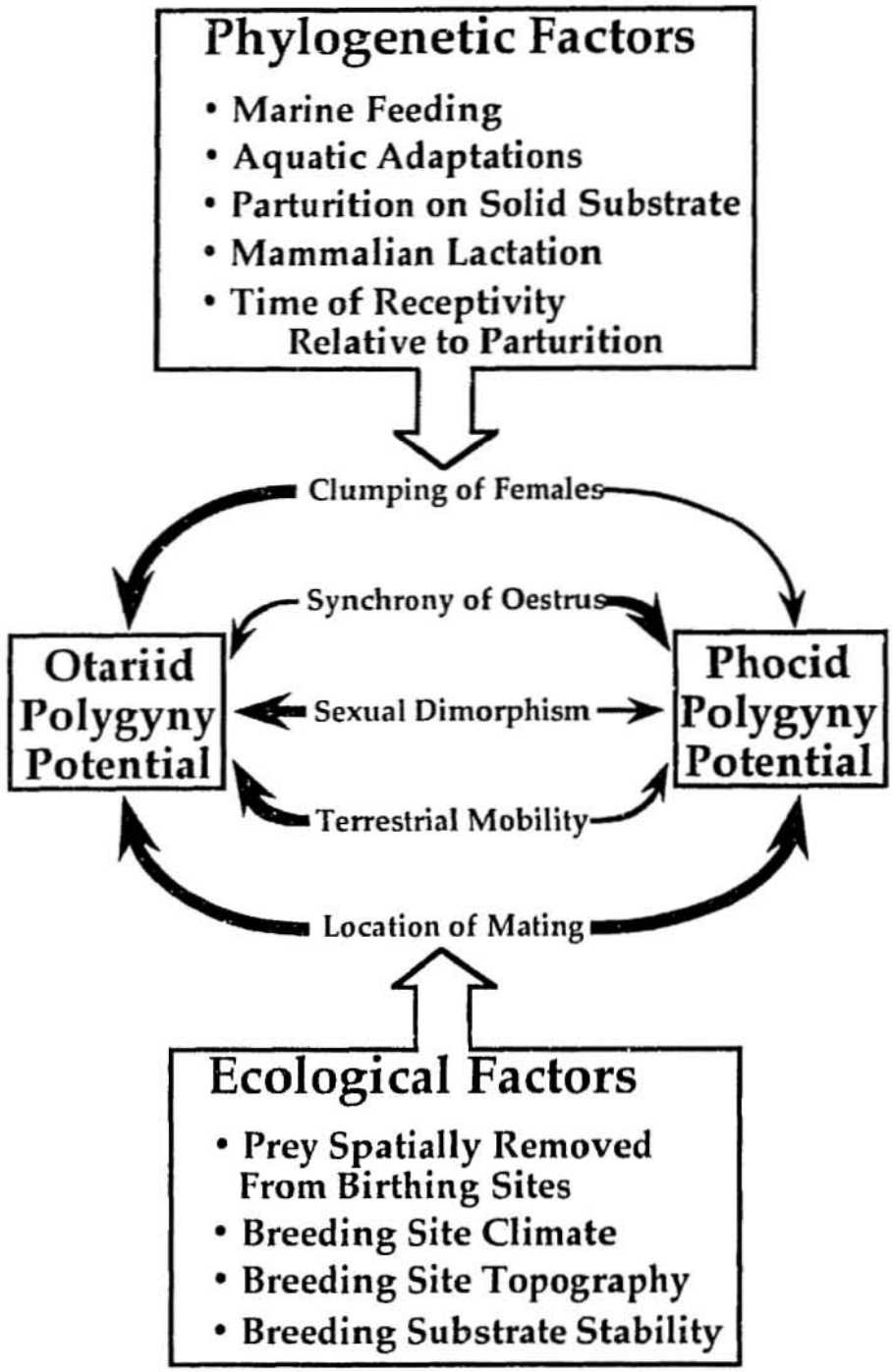

Figure 1.1: Schematic diagram of factors affecting potential for polygyny in otariid and phocid mating systems. Thicker arrows reflect increasing degree or significance of contribution to polygyny potential.

Table 1.1: The number of pinnipeds, with examples, classified in each of four polygynous mating systems (adapted from Boness *et al.* 1993).

Family	Mating System	No. of Species[A]	Example
Otariidae (15 extant species[B])	Female Defence	3	South American sea lion (*Otaria byronia*)
	Resource Defence	14	Steller's sea lion (*Eumetopias jubatus*)
	Lek	3	California sea lion (*Zalophus californianus*)
	Scramble Competition	0	
Phocidae (18 extant species[B])	Female Defence	5	Southern elephant seal (*Mirounga leonina*)
	Resource Defence	3	Weddell seal (*Leptonychotes weddelli*)
	Lek	4	Harbour seal (*Phoca vitulina*)
	Scramble Competition	1	Hawaiian monk seal (*Monachus schauinslandi*)
Odobenidae	Female Defence	1	Atlantic walrus (*Odobenus rosmarus rosmarus*)
	Lek	1	Pacific walrus (*O. rosmarus divergens*)

[A] Some species are counted more than once as their mating system does not clearly fit one category, or varies with environment.

[B] From King 1983.

Most of the research on pinniped mating systems has focused on those species which gather on land to rear their pups and also copulate on land. The relative paucity of information on the mating systems of ice-breeding seals (pagophilic seals), is probably in part due to the logistical difficulties associated with studying these species but also relates to the difficulty of observing aquatic matings. Observations of copulations plus behaviour during the breeding season not only allows us to determine what sort of mating system exists but also gives some indication of the mating success of individuals within that mating system.

In a recent and thorough review of otariid mating systems, Boness (1991), following Emlen and Oring's (1977) model, examined the phylogenetic and ecological determinants of polygyny in these animals. Female otariids are extremely gregarious during their breeding seasons, gathering on island beaches or rocky shelves, in high densities (ranging from 0.04 - 1.9 females/m^2) (Boness 1991). In general, they have a moderately asynchronous oestrus (ranging from 18 - 75 days, although most females within a species become receptive within 20 - 35 days), occurring within a few days post-partum (Boness 1991; see Gentry and Kooyman 1986 for a review of female otariid behaviour during lactation).

In addition to the spatial and temporal clustering of females, males also have an addec advantage over many other polygynous mammals. The male

otariids, like all other pinnipeds, have subcutaneous blubber stores which aid in heat conservation in the aquatic medium. During the breeding season the males can rely on this blubber as an energy store while they defend territories or females and fast (Bartholomew 1970, Jouventin and Cornet 1980, Pierotti and Pierotti 1980). Further, there is extreme sexual dimorphism in the otariids, with males being on average three times the mass of females (Boness 1991). Increased sexual dimorphism is associated with a higher degree of male polygyny. Larger males have greater potential for winning male-male fights, as well as for longer fasting durations, both of which allow large males to remain in territories for a greater portion of the breeding season and should contribute to increasing their reproductive success (Emlen and Oring 1977). All of these factors are associated with highly polygynous mating systems.

Resource Defence Polygyny:

The majority of the otariids studied exhibit resource defence polygyny (Stirling 1975a, 1983, Boness 1991). Males of these species establish territories before the females arrive on the beaches for parturition, while beach topography and temperature appear to influence the locations of females on the beaches (Gentry 1973, Marlow 1975, Trillmich 1986, Carey 1991, Francis and Boness 1991). In general, these females do not move a great deal before oestrus and any movements are predictable and associated with

thermoregulation. For example, female New Zealand fur seals (*Arctocephalus forsteri*) give birth to their pups in the austral summer (Miller 1975), when their blubber and dense fur would make heat dissipation on land problematic, as for other fur seal species (Irving *et al.* 1962). Carey (1991) demonstrated that these females change their positions on a beach to move to areas which had been modified to include shade. This suggests that males should defend territories in which there is a cooling substrate (shade or access to water) to have the greatest reproductive success, as these appear to be critical resources to females. There is evidence for some species that territorial males whose territories include water do have equal (Francis and Boness 1991), if not greater reproductive success than males with purely terrestrial territories (Heath and Francis 1983, as cited in Francis and Boness 1991; Campagna and LeBoeuf 1988b).

In general, the mating systems of phocids are not well known. Males of one phocid species, the Weddell seal (*Leptonychotes weddelli*), defend aquatic territories (Cline, Siniff and Erickson 1971; Kaufman, Siniff and Reichle 1975; Wartzok *et al.* 1989). There are two major factors which contribute to this mating strategy. First, the females gather into predictable aggregations for parturition on Antarctic fast ice, always near breathing holes and open leads in the ice (Stirling 1969, Tedman and Bryden 1979). Not long after the pups are born, females start entering the water and the amount of time they spend in

the water increases toward weaning (Thomas and DeMaster 1983). Secondly, these animals copulate underwater (Cline *et al.* 1971; Bartsh, Johnston and Siniff 1992). Therefore, it is not surprising that males compete to defend the water associated with the access points through which females must pass.

Female Defence Polygyny:

The mating system of South American sea lions (*Otaria byronia*) varies between areas. Females in Punta Norte tend to cluster into one large group, on large uniform beaches, and the mating system for this population is clearly female defence polygyny (Campagna and Le Boeuf 1988a). It is still unclear, though, whether high female density or intense male-male competition explains the female defence polygyny exhibited by this population (Boness 1991). In contrast, females at Puerto Pirámide tend to gather into smaller clusters and there is a resource (haul-out locations) defence mating system (Campagna and Le Boeuf 1988b).

Three phocid species, northern elephant seals, southern elephant seals and grey seals (*Halichoerus grypus*), have female defence polygynous mating systems (Le Boeuf 1974, Boness and James 1979, McCann 1981). Like the otariids, these phocids gather on land (except in the case of the ice breeding grey seal, Stobo and Zwanenburg 1990) for parturition and also copulate on the beaches (Stirling 1975a). However, after aggregating, the females remain

relatively sedentary (Anderson and Harwood 1985), fasting throughout their short lactation periods, and mating within a few days of weaning their pups and returning to sea. Oestrus is moderately synchronous, spanning approximately 4 - 6 weeks in grey seals (calculated from Boness and James 1979, Anderson and Fedak 1987) and 7 - 8 weeks in northern elephant seals (Le Boeuf 1974, Le Boeuf and Reiter 1988).

Grey seal males on Sable Island have been described as competing among themselves to remain near one or several females, thus establishing "tenure" in proximity to females (Boness and James 1979, Boness 1984). All tenured males have equal status, based on the outcomes of aggressive encounters, and no dominance hierarchy is established. Untenured, "transient", males spend less than two consecutive days in the same location. The east Atlantic grey seals also compete among themselves to maintain a position within the breeding colony, but do establish dominance hierarchies, with the ratio of dominant males to females ranging from 1:7 to 1:10 (Anderson, Burton and Summers 1975; Anderson and Fedak 1985; Twiss 1991). The differences between strategies employed by the two populations appears to be influenced by topography of the breeding sites. The breeding habitat of western Atlantic grey seals is much more uniform than that of the eastern Atlantic population. Females are more evenly distributed along the beaches in the western Atlantic, which could account for the lesser degree of polygyny in this population

(Boness and James 1979, Anderson and Fedak 1985, but see Twiss 1991). Boness and James (1979) argued that there is mixing between the land-breeding and ice-breeding grey seals in the western Atlantic and, because the ice habitat is less stable, the strategy employed by animals on Sable Island may be one best suited to the ice environment.

In comparison to grey seals, male elephant seals are much larger, show greater sexual dimorphism, and exhibit a more extreme degree of polygyny (*e.g.* Le Boeuf 1972, 1974, Le Boeuf and Reiter 1988). Deutsch *et al.* (1990) argued that the differences in reproductive effort between male grey seals and elephant seals may relate to the differences in male-male competition and the length of tenure, and therefore, time of fasting, on the breeding beaches. Male elephant seals arrive at the breeding beaches before the females and establish dominance hierarchies (Le Boeuf 1972, 1974, Cox and Le Boeuf 1977, Le Boeuf and Reiter 1988), competing to remain within dense female harems (McCann 1981). The males remain there through the 7 to 8-week period in which females are receptive (Le Boeuf 1974, Le Boeuf and Reiter 1988) whereas grey seal males arrive after the females (Anderson *et al.* 1975) and few remain for the entire 6 weeks that females are ashore (Anderson and Fedak 1985). Competition for mates in elephant seals, is extreme and only a few males account for the majority of copulations (Le Boeuf and Reiter 1988, Deutsch *et al.* 1990). Clearly, larger male elephant seals can benefit from their

larger size in comparison to smaller males, by having increased fighting success. Larger size also contributes to males' abilities to fast for longer periods and remain in association with females throughout the breeding period.

Lek Polygyny:

The California sea lion (*Zalophus californianus*) and Hooker's sea lion (*Phocartos hookeri*) appear to lek (Boness 1991). Boness (1991) suggests that the most likely explanations for the occurrence of leks in these two otariid species seems to be the home-range hypothesis proposed by Bradbury (1981). As females of both species move along the beaches from their pupping site to another location for mating, they come into contact with many males. It is difficult for males to defend the large areas covered by females and the female movement of these species increases the potential for extended male-male competition. In addition, California sea lion females begin foraging trips before oestrus which makes their location at oestrus less predictable. Under these conditions, males could search for mates (scramble competition) but it seems more economical for males to advertise than to search for wide-ranging females or to try to defend either territories or the females directly (Boness 1991).

The mating system of walruses (*Odobenus rosmarus*) is not clear, although environmental conditions and the behaviour of males suggest a

polygynous mating system. Females congregate into herds on ice flows in the Bering Sea (*O. rosmarus divergens*), and are attended by one or more large adult males, which remain in the water and perform displays (Fay 1982). The displays involve vocalizations (Ray and Watkins 1975), and when more than one male is present the males space themselves and continue displaying in fixed locations to which females go (Fay 1982). This is similar to behaviour seen in lekking species (Le Boeuf 1991). Fay (1982) reports aggressive encounters between males, resulting in physical injury. Generally, in a lek system one would expect less effort expended on aggression and more in display (Emlen and Oring 1977, Bradbury 1981), once the dominance heirarchy within the lek has been established. Atlantic walruses (*O. rosmarus rosmarus*) appear to be more polygynous than Pacific walruses and may defend females (Sjare 1989).

Scramble Competition Polygyny:

Scramble competition polygyny has been suggested as the mating system of Hawaiian monk seals (*Monachus schauinlandi*) (Deutsch 1985). The density of these phocid females on breeding beaches is relatively low in comparison to other land-breeding seals (Boness 1990). Further, the pupping period is relatively long in comparison to other phocids (Boness 1990) and, therefore, it is likely that oestrus is also moderately asynchronous. Kenyon and

Rice (1959) report mating activity in this species over a 4-month period. With this level of asynchronous oestrus and the dispersion of females on the breeding beaches, it is very unlikely that there would be a highly polygynous mating system, although some males may be better at finding mates than others. Males do exhibit patrolling behaviour (Deutsch 1985) which would be expected in scramble competition.

Undetermined Pinniped Mating Systems:

Little is known about mating systems of the remaining seals and, in particular, there is a paucity of data on the phocids. This is most likely attributable to a combination of factors: a majority of these species aggregate on ice for parturition, have short pup rearing seasons, and they copulate in the water. The ice habitat provides unlimited space on which females can gather for parturition, which reduces the density of animals in comparison to the islands and beaches on which temperate species pup (Stirling 1983). However, ice is an unpredictable substrate which is subject to environmental conditions and can break up quickly. This lack of stability has been implicated in the reduced duration of lactation in the ice-breeding species (Bonner 1984; Oftedal, Boness and Tedman 1987; Bowen 1991). An extreme example of short lactation is the hooded seal's (*Cystophora cristata*) 4-day lactation period (Bowen, Oftedal and Boness 1985). In addition, the lack of stability of the ice

substrate demands that seals which breed there be highly synchronous in their pupping and mating periods (Stirling 1983), as exemplified by the approximately two-week pupping periods of hooded seals and harp seals (*Phoca groenlandica*) (Bowen, Myers and Hay 1987; Stenson *et al.* 1991). Thus, it seems unlikely that ice-breeding seals would exhibit any extreme forms of polygyny (Stirling 1983). The relatively high synchronicity of oestrus coupled with reduced density of females, reduces the potential for female defence polygyny in pagophilic seals (*e.g.* Boness, Bowen and Oftedal 1988; Kovacs 1990). It also appears that the potential for pagophilic seals to have resource defence polygynous mating systems is limited. One might expect some form of scramble competition or lek system for these species.

Bartholomew (1970) suggests that it would be extremely difficult for male seals to defend an aquatic territory as these would be in a three-dimensional, boundaryless medium in which the seals are extremely mobile. This seems like a weak argument as there are many examples of both fish (*e.g.* Nursall 1977, Doherty 1983, Gross 1984) and birds (*e.g.* Dhondt and Schillemans 1983, Hatchwell and Davies 1992b) which clearly defend three-dimensional territories. Further, many fish territories do not have immediately apparent geographic boundaries identifying territory limits, and researchers rely on points of interactions between neighbouring male fish to assist them in delineating territory boundaries (*e.g.* Côté and Hunte 1989). Stirling (1975a)

further argues that the requirement for seals to return to the surface to breath would not only make defence of an aquatic territory extremely difficult, but it would make aggregation of females in such a territory unlikely. Although aggregation of females in a territory would be beneficial to resident males, it is not essential as long as females regularly pass through the defended areas.

Weddell seals (Kaufman *et al.* 1975, Bartsh *et al.* 1992) and Juan Fernández fur seals (*Arctocephalus philippii*, Francis and Boness 1991) have been found to defend aquatic territories. Male Weddell seals defend access points to the water which are likely to be used by females and thus, females would not be aggregating, but only passing through their territories. Male Juan Fernández fur seals defend water in which females do aggregate for thermoregulatory purposes. Therefore, it is most likely that if a resource essential to females is controllable through defence of an aquatic territory, then males will establish these territories, even if territory defence only ensures regular, but not necessarily prolonged, access to females. Thus, to determine the potential for polygyny in the water-mating seals, it is necessary to determine the behaviours of individual males, as well as to examine what the females are doing during lactation in areas that are apparently defended by males.

Several ecological characteristics of harbour seals during their breeding season, indicate that there is potential for polygyny to exist. Harbour seals

congregate in relatively dense aggregations on beaches and rocky outcroppings for parturition (Boulva 1975, Lawson and Renouf 1985). They appear to prefer haul-out locations which provide immediate access to deep water (Bigg 1969, Davis and Renouf 1987). Thus, there is predictable clumping of females in specific locations during the breeding period, which increases the potential for polygyny (Emlen and Oring 1977). Nonetheless, the degree of polygyny is likely to be limited as a result of two factors. Firstly, oestrus, which occurs after lactation (Bigg 1969, Bigg and Fisher 1975), appears to be relatively synchronous, based on birthing data (*e.g.* Lawson and Renouf 1985, Rosen 1990). Secondly, unlike most phocids, females and pups spend a great proportion of time in the water and haul-out for only part of the tide cycle (*e.g.* Renouf *et al.* 1981, Perry and Renouf 1988). Female movement to and from haul-out sites would make it difficult for males to defend a group of females throughout the mating period. Although female movement would make a female defence system unlikely in harbour seals, tide-related movement of females would make it possible for males to defend access points to these sites (as in the Weddell seal). Therefore, there is some potential for polygyny, from the male harbour seal's perspective.

The purpose of this thesis is to use the paternity results from DNA fingerprinting and behavioural observations of harbour seals at Miquelon to

determine the extent of polygyny and whether or not males are defending aquatic territories.

CHAPTER 2

DNA FINGERPRINTING STUDY OF A CAPTIVE BREEDING GROUP OF HARBOUR SEALS

Reproductive success is usually estimated from the number of successful copulations, eggs laid, offspring born, or young that survive to independence. Using these observable traits to assign fitness estimates to individuals can lead to erroneous estimates of reproductive success because covert behaviours, such as extra-pair copulations (EPC), intraspecific brood parasitism (IBP) and other "sneaky" reproductive strategies, occur in many species (Cooke and Mirsky 1972; Hanken and Sherman 1981; Brown and Brown 1988; Cooke and Rockwell 1988; Gibbs *et al.* 1990). As researchers observe more of these covert behaviours they are recognizing the need to use genetic markers in determining parentage and relatedness (Burke 1989, Pemberton *et al.* 1992). Two commonly used genetic markers are phenotypic characteristics such as colour morphs, and DNA products such as protein polymorphisms.

Using colour morphs as genetic markers has proven useful for determining genetic relatedness within some captive species (see for example Dewsbury 1984; Storey, French and Payne 1992) and wild populations (Cooke and Mirsky, 1972). However, colour morphs are often not precise enough to address specific questions of relatedness in wild populations. For example, the

lesser snow goose, a monogamously breeding species (Cooke and Rockwell 1988), has two plumage colour morphs, a blue phase dominant to the white (Cooke and Mirsky 1972). The existence of blue-phase goslings in nests of white-phase care-givers made it clear that these goslings arose through either EPC or IBP. Unfortunately it was not possible to distinguishing between EPC and IBP in this species, based on observations of colour morphs alone (Quinn *et al.* 1987). For colour morphs to be useful there need to be more than two morphs within a species and a simple mode of inheritance, particularly if there are several putative fathers. In addition, colour morphs can only be used to exclude potential fathers and is considered a paternity-exclusion analysis.

Protein electrophoresis has been used successfully for population differentiation, and to a limited degree for assessing reproductive success (*e.g.* Foltz and Hoogland 1981, Zweifel and Dessauer 1983, McCauley and O'Donnell 1984, Gavin and Bollinger 1985, Brown and Brown 1988, Storey *et al.* 1992). Isozymes are often inadequate genetic markers, however, for assigning parentage to individuals within wild populations (Wetton *et al.* 1987) because skewed distributions of allele frequencies result in most individuals being of one or two genotypes. This limited variation in isozymes within most populations leaves protein polymorphisms as a tool for paternity-exclusion analysis only. Also, it is not uncommon to find that there are few detectable polymorphisms within some species, which reduces the amount of information

available at the individual level. This is particularly problematic in birds (Barrowclough, Johnson and Zink 1985) and some mammals (*e.g.* McDermid and Bonner 1975, Inoue *et al.* 1990).

Paternity-exclusion analysis has successfully "caught" the erroneous assumptions in estimates of reproductive success by determining that at least one of the young was not fathered by the attending male (*e.g.* McCracken and Bradbury 1977, Hoogland and Foltz 1982). Unfortunately, paternity-exclusion analysis cannot determine the true parentage of offspring as it only eliminates potential fathers and, therefore, cannot be used as a reliable measure of reproductive success. In addition, many of the proteins must be extracted from tissues such as liver and, therefore, can only be examined post-mortem which makes this technique unsuitable for assessing lifetime reproductive success.

DNA fingerprinting is proving to be an extremely robust technique, and is being more frequently used to evaluate reproductive success, as it allows for a direct measure of genetic relatedness among individuals. Jeffreys, Wilson and Thein (1985a) first used the term DNA "fingerprint" to describe the individually unique band pattern they found after probing digested human DNA with a minisatellite discovered within the first intron of the myoglobin gene (Weller *et al.* 1984). They have created several probes, all of which are short tandem repeats of a short, unique core sequence (GGGCAGGAXG, Jeffreys *et al.* 1985b), and which detected many highly variable DNA fragments spread

throughout the human autosomal genome (Jeffreys *et al.* 1986). The hypervariability of these minisatellites results from the varying numbers of repeats of the core sequence, such that there can be a wide range in the variety of different length segments which could exist in an individual at any locus (Jeffreys 1987). Jeffreys *et al.* (1985a, b) estimated that the larger fragments in DNA fingerprints have a very low mean allele frequency (<0.04) and a high mean heterozygosity ($>96\%$). Jeffreys *et al.* (1985b) calculated the probability of two unrelated individuals sharing the same band pattern produced with one probe (33.15) to be much less than 3×10^{-11}. If two probes are used, 33.15 and 33.6, this probability drops to much less than 5×10^{-19}. If two individuals are related, then these probabilities increase to a maximum for parents and offspring. The probability that two full siblings will have the same band patterns produced with two probes is less than 1×10^{-8}. Thus, they concluded that DNA fingerprints are almost totally individual-specific and able to distinguish between members of a single family (except, of course, between monozygotic twins).

One of the most important characteristics of DNA fingerprints is the pattern of band inheritance. The hypervariable fragments are passed from parents to offspring in Mendelian fashion so that each fragment in the offspring can be found in one or the other parent but not both, except in the very rare event that a mutation occurs (in the order of 0.001-0.004 per locus per gamete

for the longest fragments, Jeffreys *et al.* 1985a, b). The technique is so robust that it can be used to reconstruct one parent's band pattern if samples from the other parent and several undisputed offspring (full-siblings) are available (Jeffreys, Brookfield and Semeonoff, 1985c). Because all bands in the offspring can be attributed to both parents, DNA fingerprinting is considered an inclusive paternity analysis whereas other paternity tests, including protein electrophoresis, are aimed at excluding potential fathers (Jeffreys 1987). This makes fingerprinting a more definitive tool than other techniques to assess paternity.

Since Jeffreys first used the term DNA fingerprinting, the technique has been applied to human samples for a variety of purposes (*e.g.* Gill, Jeffreys and Werrett 1985; Jeffreys *et al.* 1985c; Helminen *et al.* 1988, 1991, 1992; Stacey 1991). Fingerprinting has also been used successfully as a research tool with a variety of animal species. It has clear applications to population biology because it allows measurement of genetic diversity in free-ranging populations (Ellegren, Andersson and Wallin 1991; Gilbert *et al.* 1991). It can help in captive-colony management through assessment of inbreeding levels (Weiss *et al.* 1988; Ely, Alford and Ferrel 1991; Inoue *et al.* 1990, 1991). Identity of the sires in controlled breeding programs has been confirmed using DNA fingerprinting (Morton *et al.* 1987). This technique appears to have applications for the breeding of economically important domestic animal species

(Georges *et al.* 1988; Buitkamp, Ammer and Geldermann 1991). Recently, DNA fingerprinting has also been used as a tool in the protection of threatened species (Wolfes *et al.* 1991) by allowing for the identification of an individual's source population. Of most significance to this study, behavioural ecologists have turned to DNA fingerprinting as a tool with which to assess reproductive success with respect to mating systems and reproductive strategies (Burke 1989, Pemberton *et al.* 1992, Boness *et al.* 1993).

DNA fingerprinting has confirmed that data from field observations have accurately estimated relatedness or reproductive success for some species, such as willow (*Phylloscopus trochilus*) and wood warblers (*P. sibiliatrix*, Gyllensten, Jakobsson and Temrin 1990), dunnocks (*Prunella modularis*, Burke *et al.* 1989), California mice (*Peromyscus californicus*, Ribble 1991) and lions (*Panthera leo*, Gilbert *et al.* 1991). However, a majority of DNA studies are finding that there is indeed a discrepancy between the estimated and actual reproductive success of individuals within many species as a result of "sneaky" behaviours (EPC and IBP) or faulty assumptions by investigators as in: indigo buntings (*Passerina cyanea*, Westneat 1990), red-winged blackbirds (*Agelaius phoeniceus*, Gibbs *et al.* 1990), zebra finches (*Taenopygia guttata*, Birkhead *et al.* 1990) and red deer (*Cervus elaphus*, Pemberton *et al.* 1992). Thus, estimates of reproductive success may be useful for monogamous species, and those species in which the females conspicuously mate with more than one

male (the reproductive success of each male is then likely to be related to the number of copulations for each male). However, in species where females are *assumed* to mate with only one male, and their mating behaviour is relatively covert, more precise genetic measures of relatedness are necessary to determine individual reproductive success. DNA fingerprinting has the potential to satisfy this need.

Not only is DNA fingerprinting an extremely powerful technique for identifying individuals and potentially useful in examining genetic relatedness, but there are also practical benefits to field biologists in using the technique. First, the fingerprints remain constant within an individual no matter what the source of the DNA; blood, semen, or tissue (Gill *et al.* 1985, Jeffreys *et al.* 1985b). This is a particular advantage to those conducting behavioural studies of free-ranging animals as it is possible to collect a variety of tissue types, such as hairs (Higuchi *et al.* 1988) or shed skin (Hoelzel and Amos 1988, Amos *et al.* 1992), thereby eliminating the need to disturb animals under observation. A second advantage is that DNA is relatively stable.

Tissue preservation is often very difficult under field conditions, and enzyme degradation has long been a problem for isozyme analysis, which requires the use of fresh samples. In contrast, Amos (1989) and Amos and Hoelzel (1991) have described simple methods for preserving tissues for DNA analysis, without refrigeration, within saturated salt solutions. These methods

have proven to maintain nuclear and mitochondrial DNA intact at room temperatures for up to two years, thereby reducing tissue preservation problems.

Many behavioural ecologists are turning to DNA fingerprinting to address questions of paternity and genetic relatedness. There are some authors who have reservations about using the technique for determining genetic relatedness, although all agree that DNA fingerprinting can be used to determine parentage. Lynch (1988) has argued that it is impossible to know from which loci minisatellites originate: Two minisatellites of equal length will appear in the same location on two separate gels. In this case it is difficult to determine if the bands represent the same locus, indicating some degree of relatedness, or similarly-lengthed alleles from two different loci, where no relatedness is implied. For this reason, it is impossible to know if an individual is homozygous or heterozygous for a particular allele and, therefore, he argues that it is impossible to know the proportion of shared genes between individuals (Lynch 1988). Thus, baseline estimates of band-sharing between unrelated individuals in a population should be determined before using this technique to examine relatedness between individuals (Lynch 1988, Cummings and Hallett 1991).

It is also difficult to develop statistical models for determining genetic relatedness from DNA fingerprints (Lynch 1988). Because many of the smaller

restriction fragments are run off the gel, to obtain sufficient separation of the larger fragments, there is incomplete visualization of minisatellites and, therefore, the complete genome is not represented. Furthermore, some of the minisatellite loci may be linked so that some fragments may be passed together which would reduce the number of informative bands. All of these potential problems lead Lynch (1988) to caution against using DNA fingerprinting for determining genetic relatedness beyond first order relatives (parents and offspring). For these reasons, a pedigree analysis of a large family (both parents with ten or more offspring, Burke 1989) should be completed to detect linked alleles before using DNA fingerprinting for determining genetic relatedness within a population of that species (Hoelzel, Ford and Dover 1991).

Pedigree analyses, using the Jeffreys' probes, have been carried out for a few species: honeybees (Blanchetot 1991), humans (Jeffreys *et al.* 1986), mice (Jeffreys *et al.* 1987), dogs and cats (Jeffreys and Morton 1987), house sparrows (Burke and Bruford 1987; Wetton *et al.* 1987), dunnocks (Burke *et al.* 1989), indigo bunting (Westneat 1990) and zebra finches (Birkhead *et al.* 1990). A majority of the bands in all of these species (except the mouse) were found to segregate independently and the probability of including a non-relative as the father, using the two probes, ranged from approximately 10^{-5} to 10^{-6}. However, in the mouse, up to 10 fragments appear to be linked (Jeffreys *et al.* 1987) which reduces the number of informative bands and, therefore, would

increase the probability of misassigning paternity and miscalculating relatedness. These results underline the importance of conducting a pedigree analysis before using DNA fingerprinting to assess genetic relatedness.

Pedigree analysis is more easily accomplished with captive groups of smaller species in which many offspring are born per breeding cycle, and in which paternity can be controlled. For larger, free-ranging mammals, it can be quite difficult, if not impossible, to have a large enough family group composed of many full-siblings (c.f Hoelzel *et al.* 1991). However, DNA fingerprinting can be used to determine paternities within these species. Determining parentage depends on the identification of alleles which are not attributable to the known parent and, therefore, coming from the other parent and, thus does not involve any statistical techniques (Lynch 1988). For this reason, DNA fingerprinting does have significant applications in the field of behavioural ecology.

At the time of this study DNA fingerprinting had not been attempted with any seal species[1]. The objective of this study was to first determine if DNA fingerprinting would produce individually-unique band patterns for harbour seals by using a captive breeding group. If so, then the technique would be used to conduct a paternity analysis to determine if all bands visualized in the offspring are present in the father, the mother or both.

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[1] Since completion of this study, DNA fingerprinting has been used successfully with hooded seals (McRae and Kovacs 1991) and harbour seals (Harris *et al.* 1991).

METHODS

Subjects:

A captive breeding colony comprised of six seals (two adult males, one adult female and three of her offspring) was used for this preliminary analysis. The animals are housed in an outdoor facility at the Ocean Sciences Centre, St. John's, Newfoundland. Prior to collecting blood, each seal was restrained in a box, large enough to fit comfortably over the seal such that its hind flippers protruded through a rectangular hole cut in one end of the box. Blood was drawn from the hindflipper of each seal following the procedure described by Geraci (1971), using 7ml EDTA Vacutainers. One tube was collected from the adult female and each of her offspring. Two tubes were drawn from each of the adult males. Immediately following blood sampling, the blood was transferred to labelled, sterile 50ml Corning tubes and stored at -40°C.

DNA Fingerprinting:

All of the laboratory procedures for DNA fingerprinting were conducted in 1989 at the Dept. of Genetics at Cambridge University under the direction of Dr. W. Amos.

DNA Extraction:

Once blood samples had thawed at room temperature, each was mixed by stirring with a glass rod and 0.6ml of each sample was transferred to a 1.5ml Eppendorf tube. To each sample, 0.65ml of 7 X Digest Solution (see Appendix I) and 0.25mg of Protease-K was added. The solution was stirred with a glass rod and then heated in a water bath at 65°C for 15 minutes. Samples were transferred to a 37°C heating block and left for a minimum of 1.5 hours.

To each sample, 0.5ml phenol was added and mixed. Samples were left at room temperature for 5 minutes, restirred and spun for approximately 15 seconds. Chloroform (0.6ml) as added to the supernatant and then the solution was mixed by gentle rocking and left it at room temperature for 5 minutes. The supernatant was divided into aliquots and an equal volume of 5M LiCl was added to each. Following gentle mixing, samples were placed at -20°C for a minimum of 30 minutes and then spun for 5 minutes.

The supernatant was divided into 450*ul* subsamples, leaving any sediment behind. I added 1.0ml 100% ethanol to each sample and then rocked them gently. Samples were returned to -20°C for a minimum of 1.5 hours and then, following spinning at full speed (13,000 *g*) for 5 minutes, the supernatant was poured off leaving a pellet of genomic DNA.

The pellet was washed with 1.0ml 70% ethanol, spun for 5 minutes and then the supernatant was discarded. Pellets were dried under vacuum for 30 minutes and then resuspended in 100*u*l of TE (see Appendix I). Like samples were combined after 5 minutes of heating in a 65°C water bath.

Digestion:

Samples, 5*u*l sample plus 5*u*l loading buffer (TE:Tacon, 2:1), were run on a 0.6% agarose in TBE test gel for 2 hours at 100V. Gels were visualized using EtBr/UV light and photographed (see Figure 2.1a for example) for a comparative estimation of DNA concentrations in samples. Up to 100*u*l of TE was added to samples to balance concentrations of DNA.

To determine which restriction endonucleases would lead to the clearest DNA fingerprints, samples from captive harbour seals were divided into 4-17*u*l subsamples. These were digested with *Hae* III, *Hinf* I, *Alu* I and *Dde* I. *Dde* I was found to give the clearest fingerprints and most polymorphic bands (Figure 2.2) and, therefore, was used for all digests.

17*u*l of each sample was digested overnight in a block heater at 37°C with 1*u*l High Incubation Buffer, 1*u*l Spermidine and 0.5*u*l enzyme. Complete digestion was confirmed by running another test gel (see Figure 2.1b for example). Digested samples were precipitated using 0.1 volumes 5M NaAc plus 2.5 volumes 100% ethanol at -20°C for a minimum of 30 minutes,

spinning for 5 minutes and pouring off supernatant. The DNA pellet was washed with 70% ethanol, dried under vacuum and resuspended in TE.

Separation:

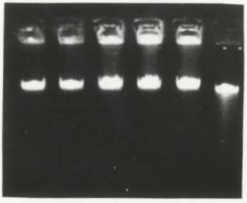
DNA fragments were separated on 27cm 1.0% agarose in TBE gels at 50V for 2 days or until 2Kb fragments had run off the gel.

Blotting:

Separated DNA fragments, while still in the gel, were depurinated by incubation in 0.25M HCl for 20 minutes. Then, if fragments were to be blotted onto nitrocellulose filters, the gels were submersed in denaturing solution (Appendix I) for 45 minutes, transferred to neutralizing solution (0.5M Tris, pH 7.0) for 45 - 60 minutes. To ensure absorption of liquids, the filters were given a short wash in distilled water after which the fragments were vacuum blotted, using LKB 2016 Bromma Vacugene vacuum blotting pump and unit, onto the nitrocellulose filters in high salt conditions (20 X SSC). When Hybond-N filters were used for blotting, and then the gels were submersed in denaturing solution for 10 - 15 minutes, followed by blotting using the vacuum blotter and alkaline transfer buffer. Filters were neutralized by washing in 0.5M Tris, pH 7.0.

Upon completion of blotting, filters were neutralized with 2 X SSC and then DNA was fixed onto the filters by UV cross-linking.

a)



b)

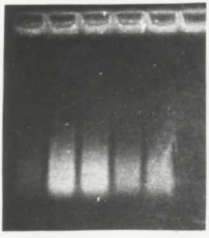


Figure 2.1: Photographs of seal DNA in 0.6% agarose in TBE test gel run for 2 hours at 100V a) undigested to estimate relative quantities of DNA in samples and b) completely digested (stained with EtBr and visualized with UV light).

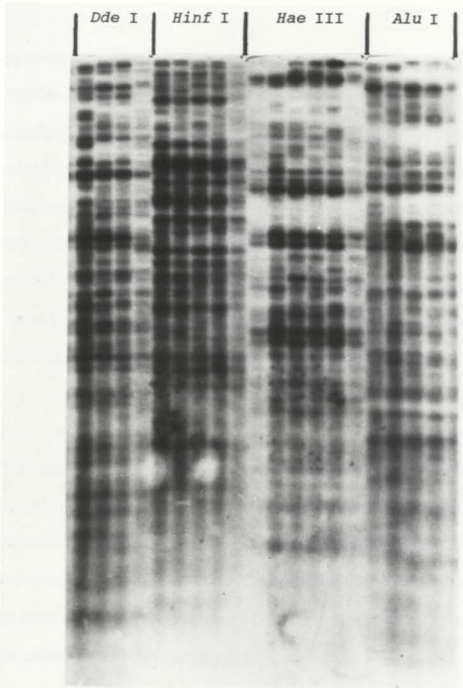


Figure 2.2: Autoradiogram of seal samples digested with *Dde* I, *Hinf* I, *Hae* III and *Alu* I.

Probes:

The polycore probes 33.15 [$(AGAGGTGGGCAGGTGG)_{29}$] and 33.6 [$[(AGGGCTGGAGG)_3]_{18}$] (Jeffreys *et al.* 1985b) were cloned into the M13 vector (supplied by Dr. W. Amos, Dept. of Genetics, University of Cambridge, Cambridge, U.K.). The single-stranded M13 and insert were labelled with ^{32}P through primer extension (Wells 1990). The DNA was digested with *Pst* 1 for 33.15 and *EcoR* 1 for 33.6 and then ethanol-precipitated to remove unincorporated nucleotides. The radioactively-labelled probe was resuspended and run in a 1.0% low melting point (LMP) agarose gel which was then exposed for 2 min on X-ray film to localize the labelled insert band.

Hybridization:

Filters were pre-hybridized by submersion and rocking in pre-hybridization solution (Appendix I) for two hours at 60°C. The radio-labelled probe, suspended in LMP agarose, was added directly to the filters and allowed to hybridize overnight at 60°C[2]. Filters were washed (Appendix I) twice by rocking at 65°C for 15 minutes, to remove excess probe and then filters were allowed to partially dry, followed by wrapping in a clear plastic wrap.

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[2] Prepared by Dr. W. Amos, Dept. of Genetics, University of Cambridge, Cambridge, England.

Autoradiograms:

Kodak X-ray film was used for autoradiograms. Film, intensifying screens and filters were placed in cassettes and left in -70°C for exposures ranging from four hours to one week, depending on the radioactivity of each filter. Autoradiograms were developed using a Fuji RG II film processor.

Removal of Probes:

Following autoradiography, the first probe was removed from the filter to allow for rehybridization with the second probe. The filter was washed in an alkaline solution (0.4M NaOH) at 45°C for 30 minutes and then neutralized at 45°C for 30 min, with one change of neutralizing solution (Appendix I). After a brief rinse in distilled water the filter was ready for re-hybridization.

Band Scoring:

Autoradiograms were inspected by eye using a light table. The mid-point of each scorable band was marked on a piece of acetate taped over the autoradiograms. Two readers counted the scorable bands to test for reliability of the main reader. Bands were scored by measuring the distance that the band had migrated. Two bands in adjacent lanes, were considered identical if they had migrated to within 0.5mm of each other (Westneat 1990) and were of comparable intensity (Burke and Bruford 1987).

Bands found in the offspring but not their mother were considered to be paternally-derived bands. Some of the paternal bands were common among males so it was necessary to identify informative (or diagnostic) bands that were found in only one adult male. The bands of the adult males were then examined to determine if either of the males had all paternal bands found in each of the young. In this way all paternities were documented.

Total number of scorable bands was recorded for each individual and the number of identical bands was recorded in a pair-wise fashion between individuals for calculation of the band sharing coefficient (D) using the equation:

$$D = \frac{2N_{AB}}{(N_A + N_B)}$$

where N_{AB} is the number of bands in common between individual A and individual B, and N_A and N_B are the total number of scorable bands in A and B's fingerprints, respectively. Band sharing coefficients for mothers, pups and assigned fathers were calculated.

RESULTS

There was a mean of 95.5% agreement (range = 90.6 - 100%) between the two readers' counts of total scorable bands in fingerprints produced with probe 33.15 and 90.2% agreement (range = 85.7 - 95.2%) for those produced with probe 33.6.

Banding Patterns:

Both probes revealed banding patterns that varied between individuals (Figure 2.3). Significantly more bands were detected with probe 33.15 than with probe 33.6 ($t = 8.05$, d.f. = 10, $p < 0.05$) (Table 2.1). Some of the bands visualized using probe 33.15 were also visualized by probe 33.6 (Table 2.1). The proportion of scored bands detected by both probes was $\bar{x} = 0.14 \pm 0.02$ (sd).

All scorable bands present in the offspring can be found in either the mother's or father's (see below) band patterns which indicates Mendelian inheritance of these loci.

Table 2.1: Total number of scorable bands detected in each harbour seal and number of bands common between probes 33.15 and 33.6.

	Probe		
	33.15	33.6	Common
Female	32	21	8
Offspring 1	35	17	6
Offspring 2	34	20	6
Offspring 3	29	17	6
Male 1	26	15	6
Male 2	30	17	8
$\bar{x}$	31.0	17.8	6.7
sd	3.1	2.0	0.9

Band Sharing:

Band sharing coefficients between the three, presumably unrelated adults, the female and her three offspring, and the two adult males and the offspring were calculated for each probe (Table 2.2).

The mean band-sharing coefficient between the mother and her three offspring, based on the fingerprints for both probes combined, is 0.59 (sd = 0.13, n = 6) which is similar to the expected value of 0.5. The mean band-sharing coefficient between the father (M2) and his two offspring (O1 and O3) (see below) were 0.63 (sd = 0.10, n = 2) for probe 33.15 and 0.46 (sd = 0.14, n = 2) for probe 33.6 and an overall average of 0.61 (sd = 0.12, n = 4) for the combined probes. This value is higher than the expected value of 0.5.

Band-sharing coefficients between the pups and the unrelated male (M1) (probes combined: $\bar{x}$ = 0.44, sd = 0.11, n = 6) were similar to those between the three unrelated adults (probes combined: $\bar{x}$ = 0.44, sd = 0.11, n = 6).

The average band-sharing coefficients for the presumably unrelated adults, using both probes, was significantly less than those of the mother and her offspring (one-tailed t-test: t = 1.81, d.f. = 10, $p < 0.05$).

Paternity Analysis:

Two readers analyzed paternity independently by locating paternal bands and identifying which of these were informative bands (not originating from the mother and only existing in one of the two adult males) (Table 2.3). There was complete agreement between the readers in assigning paternities, despite some variability in the number of paternal and informative bands counted (which may be attributable to practice effects). Paternities of offspring O1 and O3 were attributed to M2 as his fingerprints had all informative bands. Informative bands found in O2's fingerprints were in neither male (Figure 2.3) and, therefore, paternity was presumed to be attributable to an adult male that was no longer in the colony.

Table 2.2: Band-sharing coefficients between harbour seal offspring and their mother as well as the two adult males for each probe.

	33.15			33.6		
	F	M1	M2	F	M1	M2
O1	0.72	0.49	0.74	0.47	0.38	0.65
O2	0.67	0.43	0.50	0.68	0.23	0.32
O3	0.68	0.59	0.64	0.37	0.50	0.41
$\bar{x}$	0.69	0.51	0.63	0.51	0.37	0.46
sd	0.02	0.07	0.10	0.13	0.11	0.14

Table 2.3: Assigned father for each pup based on the number of paternal bands (PB) held in common between pups and males M_1 and M_2 in fingerprints produced using probes 33.15 and 33.6.

Probe	Pup ID	No. of PB	M_1	M_2	Father
33.15	O_1	5	2	5	M_2
	O_2	7	1	2	?
	O_3	3	2	3	M_2
33.6	O_1	2	0	2	M_2
	O_2	2	0	1	?
	O_3	2	0	2	M_2

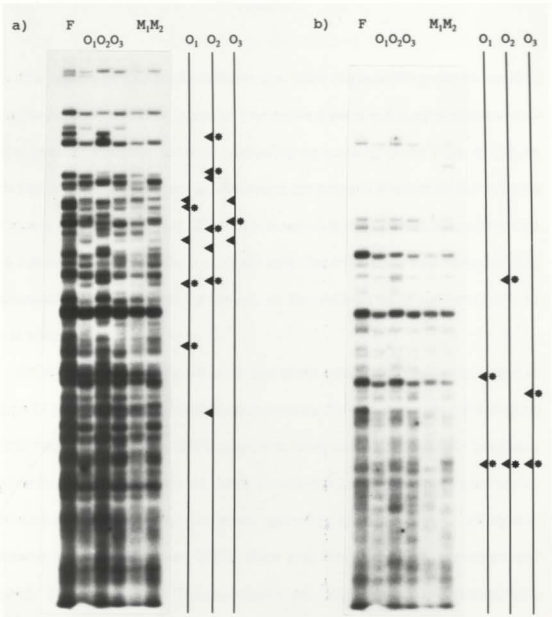

Figure 2.3: DNA fingerprints of harbour seal offspring (O_1 - O_3), their mother (F), and potential fathers (M_1 and M_2) produced with probe a) 33.15 and b) 33.6. Paternal bands are indicated by ◀.
Diagnostic paternal bands are indicated by ●.

DISCUSSION

The results of this study indicate that DNA fingerprinting can be used to determine paternity in harbour seals. The readers were in full agreement on the identity of each offspring's father, including concurring in the case of O2, in which the informative paternal bands where not present in either of the existing adult males. At the time that O2 was conceived there were three adult males in the colony and, therefore, it is likely that the third male had fathered O2. Unfortunately this could not be tested, as the male is no longer available to provide samples.

Although there was no effort to systematically collect behaviour data in this part of the study, anecdotal observations made it apparent that the finding that M2 had fathered O1 and O3 could not have been predicted on the basis of behavioural observations as both males exhibited common pre-mating behaviours in the tank, such as: water slapping, bubble-blowing, rolling and aggressive interactions (Allen 1985, Beier and Wartzok 1979, Venables and Venables 1957, 1959). These behaviours, plus aggressive interactions between the males (vocalizations, fighting, chasing and biting), started just prior to parturition and continued until post-weaning. In addition, M1 was seen copulating with the female on the bottom of the tank prior to the birth of O1, whereas M2 and the third male were never seen copulating with the female.

Although Harris, Young and Wright (1991) did not report witnessing copulation, they concluded that male harbour seal pre-intromission behaviours could not be used as reliable indicators of reproductive success. This study confirms their conclusion and also demonstrates that copulation cannot necessarily predict reproductive success.

It is apparent from the results of this study that the female harbour seal copulated with more than one male in a single breeding season. She was witnessed copulating with M1, but the pup produced during the following breeding season was fathered by M2. It is not surprising to find that our captive female mated with more than one male, as female phocids of other species have been witnessed copulating with several different males (*e.g.* Boness and James 1979, Le Boeuf and Mesnick 1990, Campagna *et al.* 1993). Nonetheless, this is the first time that multiple matings within a breeding season has been demonstrated in harbour seals. What effect females mating with several males has on male reproductive success has not been tested in any seal species to date.

Based on the results of this study, it would appear that mating-order may affect male harbour seal reproductive success and may relate to male mating behaviour, as has been found in some other animal species. There is a first-male advantage in Belding's ground squirrels (*Spermophilus beldingi*, Hanken and Sherman 1981) and the 13-lined ground squirrel (*S. tridecemlineatus*, Foltz

and Schwagmeyer 1988), in which the first males to mate sire a significantly greater number of the offspring in a litter. In the 13-lined ground squirrel, it is thought that first order mating advantage is related to better male searching abilities and contributes to the success of males in a scramble competition mating system. In contrast, male success in Idaho ground squirrels (*S. brunneus*) is related to mate guarding following copulation which reflects the increased dispersion of, and thus difficulty in locating, females in the population (Sherman 1989). Interestingly, mating order advantages vary with the approach of ovulation in golden hamsters (*Mesocricetus auratus*). Early in oestrus in this species, there is a second male mating advantage, but as ovulation approaches the first male to mate has the advantage. Therefore, mating order and timing of mating appear to have a significant affect on the reproductive success of males (Huck *et al.* 1986) and explain why dominant male golden hamsters guard mates mainly in the early part of the receptive period (Lisk *et al.* 1989). In the harbour seal, mating order and timing of mating relative to oestrus may also affect male reproductive success. It is likely that a male will have increased reproductive success if he is able to mate with females at the time of oestrus and this advantage may be reflected in the mating behaviour of males.

Band-sharing coefficients between the mother and her offspring (0.59) and the father and his offspring (0.61) were within, but slightly above, the

expected range of 0.5 (Wetton *et al.* 1987), and higher than that found for the presumably unrelated adults (0.44). These results suggest that DNA fingerprinting could be used for determining genetic relatedness beyond parentage in harbour seals although a more thorough pedigree analysis would be necessary to determine if linkage is likely.

CHAPTER 3

ASSESSING PATERNITIES OF HARBOUR SEAL PUPS AT MIQUELON USING DNA FINGERPRINTING

Inherent in discussions of mating systems is the assumption that individuals will behave in a manner which is most likely to increase their fitness. Females are expected to maximize their fitness by mating with "superior" males and ensuring survival of their offspring, while males, particularly if they are not required to help in care of the young, will compete for access to as many females as possible (Emlen and Oring 1977, Clutton-Brock 1989, Boness 1991, Davies 1991, Le Boeuf 1991). Assessing male fitness within mating systems has been problematic in wild populations as there have been no direct measures available. In the past, researchers have relied on observations of copulations as a measure of male reproductive success, but this is difficult in phocids because 15 of the 18 species copulate underwater. With the advent of molecular techniques, such as DNA fingerprinting, it is now possible to determine male fitness (Burke 1989) of these species through inclusive paternity testing, and to use this information to assess the number of females with which individual males successfully mate.

Harbour seals are extremely wary (Renouf *et al.* 1981, except see Boness *et al.* 1992), and they mate aquatically (Allen 1985), which has made

it difficult to determine their reproductive behaviour. Therefore, little is known about their mating system (Stirling 1975a, 1983, Sullivan 1981, Le Boeuf 1991). Harbour seals are only slightly dimorphic with adult males being approximately six percent longer (McLaren 1993) and 34% heavier than females (Bigg 1969). Sexual dimorphism is more extreme in the highly polygynous elephant seals. Southern elephant seal males are estimated to be up to ten times the weight of breeding females (Ling and Bryden 1981), while northern elephant seal males are three times the weight of females (McGinnis and Schusterman 1981). The west Atlantic grey seal males are twice the size of females (Bonner 1981), ranging from 170 to 310kg. The males of all three of these polygynously mating phocid species also have enlarged snouts. Although the snout of male greys seal is fixed, while those of male elephant seals can be expanded, it is apparent that these snouts are used in visual signals between males during the breeding season (Miller and Boness 1979). Harbour seals do not have any conspicuous secondary sex characteristic.

Behavioural data gathered on male harbour seals during the breeding season indicate that there is competition between males for access to females (Sullivan 1981, 1982, Davis and Renouf 1987, Chapter 4). Thus some males may mate with more than one female and a low level of polygyny is suspected (Bigg 1969, Sullivan 1981, Stirling 1983, Chapter 4). Unfortunately, it is difficult to determine how many individuals each seal is mating with as mating

has rarely been witnessed (Allen 1985). The number of females hauling out within a defended area may not be a reliable indicator of reproductive success of territorial males (Boness *et al.* 1993). For example, territorial male red-winged blackbirds gain more than 20% of their reproductive success through extra-pair copulations with females in neighbouring territories (Gibbs *et al.* 1990).

Male Weddell seals defend aquatic territories around breathing holes and open ice leads near lactating females (Kaufman *et al.* 1975, Hill 1987), however, females appear to mate with males away from the locations in which they reside (Testa, as cited by Boness *et al.* 1993). Harbour seals also mate in the water and, because copulations are rarely witnessed, it is uncertain if copulations occur near to the haul-out areas. For this reason, the best method with which to assess male harbour seal mating success, and thus determine the level of polygyny, would be DNA fingerprinting (Boness *et al.* 1993).

There appear to be two principal areas in which behavioural ecologists are employing DNA fingerprinting: in identifying parentage (usually paternity), and in determining degree of relatedness among individuals in a population. Most agree that the tool is extremely robust for determining parentage. The purpose of this study is to use DNA fingerprinting to determine paternities of pups within one haul-out location at Miquelon, in which particular males are regularly seen. Information on paternity will be used to determine if the

behaviour of particular males which have fathered pups differs from that of males which have not fathered pups.

METHODS

Study Sites:

Between May and August each year a herd of approximately 600 harbour seals congregate in the Grand Barachois of Miquelon (45° 45'N and 56°14'W), a French island 19 kilometres from the southeastern coast of Newfoundland, Canada. The Barachois is a large tidal lake with sandbars throughout its centre which become exposed as the tide ebbs. The seals gather on these sandbars as the water falls and remain there until the water reaches the high tide mark or a disturbance occurs (Renouf *et al.* 1981).

Blood Samples:

We caught seals for tagging and blood samples during the last two weeks of lactation (20 June - 03 July) in 1985 to 1989, when pups were more independent and we would be less likely to cause separation of mother-pup pairs. Adult seals were physically restrained in nets strung between a pair, two-metre aluminum poles which were pinned at one end. To reduce the disturbance of the animals on their haul-out sites, nets were held across access channels, catching seals as they swam away from the beach. Efforts were made to keep mother-pup pairs united during all stages of catching, sampling and tagging. Generally, only one pair was caught at one time and, therefore,

there were few opportunities for confusing mothers and pups. Further, females' reactions to pups were carefully monitored to ensure that mothers were kept with their own pups. Displaying males (VM, BM, CM) were corralled into nets in the water and then dragged ashore for sampling. Non-displaying males (SS, NE), hauled out on the sand were caught by sneaking up on them from the water's edge and throwing a net over them.

Tagging began in 1984 with 14 weaners (pups weaned in that breeding season) tagged that year. In the following years all seals caught were tagged with a Nasco cattle ear tag placed in the webbing of the hindflipper; left for males and right for females. A different tag colour was chosen each year so that immature animals could be aged by tag colour alone.

A total of 108 tags were placed between 1984 - 1988 (Appendix II). A summary of tag placements and re-sightings are reported in Appendix II. Of these, five have been returned by fisherman and one tagged pup was found dead in the tagging area later in the same summer. Tags were placed on two different displaying males and both males lost their tags within 5 days of tag placement. One was tagged in 1985 and the other in 1988. The first male was re-tagged in 1987, when he had been forced to an adjacent displaying area and displayed less frequently. He still had his tag in 1990. As a result of the high probability of tag loss in displaying adult males, males were identified on the basis of scars and pelage patterns on the head, face and necks.

Blood was drawn from the hindflipper of each seal following the procedure described by Geraci (1971), using 7ml EDTA Vacutainers. One tube each was collected from mothers, pups and other seals except adult males from which a minimum of two and a maximum of four tubes were taken. Taking four tubes ensured that there were sufficient samples to run males on multiple gels. Immediately following blood collection, each seal was tagged. Each vacutainer tube was labelled with the date, sex of the seal, and tag number, and in the case of mothers and pups, the pup tag number and mother tag number, respectively.

Following blood sampling, pups were brought physically close to their restrained mothers, and the females' reactions were observed to ensure that appropriate pairs were being reunited. In no case was a female aggressive toward the proffered pup and, therefore, we were confident that no pups were mismatched with mothers.

Paternity Analyses:

All blood samples were stored in the field for a maximum of three days at temperatures ranging from 4°C to 10°C. Blood samples were transported to a municipal freezer where they remained at -40°C until the field season ended, at which time they were transported to a -40°C walk-in freezer at the Ocean Sciences Centre, Newfoundland.

Samples collected in 1985-1987 were spun with a hand-crank centrifuge so that serum and red cells could be separated for later protein electrophoresis. Red cell protein polymorphisms were to be used as a method of assessing paternity, but degradation of proteins in field samples was too severe to allow for analyses. White cells from these samples were discarded. Blood samples collected in 1988-1989 were frozen as whole blood at -40°C.

DNA Fingerprinting:

The procedures for DNA fingerprinting were the same as those described in Chapter 2.

Band-sharing coefficients for 13 mothers and pups were calculated. As few paternities were assigned and there were too many sample lanes separating males and some pups, band-sharing coefficients for males and pups were not calculated. Paternal bands in the DNA fingerprints of 13 pups were compared with those of the 5 adult males to assign paternities (for examples see Figure 3.1).

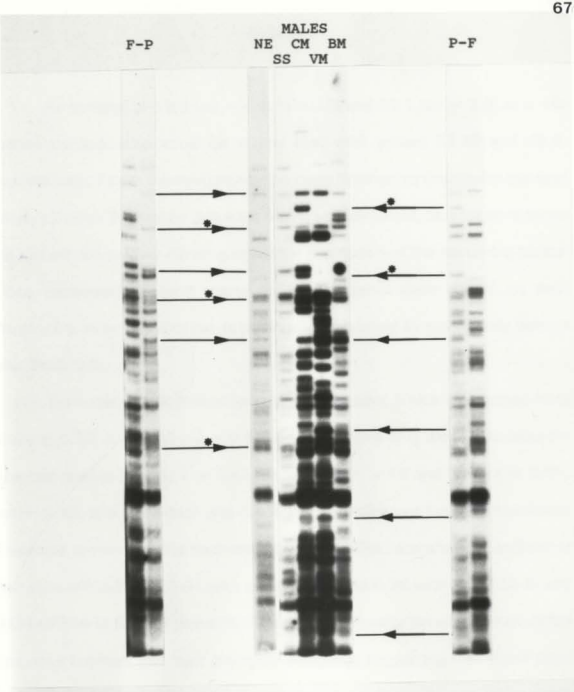

Figure 3.1: DNA fingerprints of adult males and two mother-pup pairs produced with probe 33.15. Paternal bands for pup 63 (left) and pup 101 (right) are indicated by ←. Diagnostic paternal bands are indicated by *.

RESULTS

An average of 18.9 (sd = 4.6, n = 18) and 11.1 (sd = 2.0, n = 18) bands per individual could be clearly read with probes 33.15 and 33.6, respectively. Fewer bands were read on these fingerprints than in the previous study (Chapter 2) because gels were run for slightly longer, causing more bands to run off the gel but allowing for better separation of the remaining bands. Also, conservative cut-off points for reading bands were chosen on each fingerprint, to ensure that bands in all individuals could be read clearly (except see Table 3.2).

Band-sharing coefficient for mothers and pups (Table 3.1) ranged from 0.04 to 0.78, $\bar{x}$ = 0.50 (sd = 0.17, n = 26), combining those calculated for the two probes (33.15; $\bar{x}$ = 0.45, sd = 0.15, n = 13 and 33.6; $\bar{x}$ = 0.61, sd = 0.15, n = 13) which was not appreciably different from the coefficient expected for parents and their offspring, 0.50. The band-sharing coefficients for pairs M104/P105 (0.04 with probe 33.15 and 0.36 with probe 33.6) and M244/P246 (0.28 with probe 33.15) were much lower than would be expected between mothers and their biological offspring, suggesting that these pups were being fostered. Therefore, band-sharing coefficients were recalculated excluding their scores. The average band-sharing coefficient became 0.56 (sd

= 0.11, n = 22) ($\bar{x}$ = 0.51, sd = 0.08, n = 11 and $\bar{x}$ = 0.66, sd = 0.11, n = 11, using probes 33.15 and 33.6, respectively).

Paternities were assigned based on results from autoradiograms 4 (gel 2), 5 (gel 3) and 6 (gel 6) (see Figures 3-1). No effort was made to assign paternities for pups 105 nor 246 as it was not clear that these pups were with their biological mothers. Based on informative bands, paternities could be assigned for only four of the remaining 11 pups: 54, 63, 101 and 128 (see Table 3.2).

The number of paternal bands in common between pups whose fathers were not in the samples, were counted to determine if they may have had the same fathers (Table 3.3). Comparisons were made between pups run on the same gel such that paternal bands in pups 109, 112 and 127 were compared and paternal bands in pups 249, 238 and 216 were compared. Both of the fostered pups were excluded from the comparisons. No two pups shared their paternal bands and in only one comparison was there even one band in common. Pup 216 had a total of 11 paternal bands and pup 238 had a total of nine paternal bands. They shared one paternal band which gives a paternal band sharing coefficient of 10 % which is lower than the 50 % expected in Mendelian inheritance patterns. Pups 119 and 127 were both run on gel 4 and a comparison of their paternal bands on that gel indicated that they had no

paternal bands in common. Therefore, pups run on a single gel and whose fathers were not in the sampled males, did not appear to share fathers.

Table 3.1: Band-sharing coefficients (BSC) calculated for harbour seal mothers (M) and pups (P) from DNA fingerprints produced with probes 33.15 and 33.6.

	Probe			
	33.15		33.6*	
ID	Total bands	BSC	Total bands	BSC
M53/P54	25/23	.50	13/9	.64
M64/P63	25/21	.43	12/11	.78
M102/P101	23/23	.58	13/13	.62
M104/P105	23/23	.04	13/9	.36
M110/P109	12/23	.63	--	--
M111/P112	18/24	.62	7/11	.78
M121/P119	15/14	.55	--	--
M126/P127	25/28	.43	14/22	.50
M130/P128	12/18	.40	--	--
M244/P246	23/20	.28	--	--
M250/P249	16/21	.49	--	--
M252/P238	16/18	.41	--	--
M263/P216	14/19	.55	--	--

* Some fingerprints produced with probe 33.6 were too faint to calculate band sharing coefficients.

Table 3.2: Paternity assignment, based on the number of paternal bands (PB) common between pups and males, using probe 33.15.

YEAR	PUP ID	GEL NO.	PB	MALES					ASSIGNED FATHER
				VM	BM	CM	SS	NE	
1985	54	3	5	5	3	3	3	3	VM
	63	3	6	5	5	6	4	5	CM
1986	101	6	6	4	3	6	2	1	CM
	109	6	8	7	5	5	6	5	None
	112	6	10	7	5	6	7	5	None
	119	2	8	5	6	6	3	-	None
	127	6	10	4	5	5	6	5	None
	128	2	7	3	4	4	7	-	SS*
1989	249	3	5	3	3	1	4	-	None
	238	3	14	6	7	6	8	-	None
	216	3	9	3	1	4	3	-	None

* Six informative bands are clearly shared between SS and pup 128. The seventh band is difficult to read but appears to be shared.

Table 3.3: The possibility that pups whose fathers were not in the sample may have had a common father based on the number of paternal bands in common between pups run on the same gels.

GEL #	PUP ID	127	109	COMMON FATHER
6	112	0	0	No
	127		0	No
3		238	216	
	249	0	0	No
	238		1	No

DISCUSSION

Three of the five males included in this study, CM, VM and SS, fathered pups. Neither BM nor NE fathered any of the pups. All females and their pups were caught in the area in which males BM, VM, CM, and SS were regularly seen (see Figure 4.5). Male NE was never seen in this area and regularly hauled out with another haul-out group. Therefore, it is not surprising that he did not father any of the pups. BM, CM, VM, and SS were all caught in the study area and VM was the male in closest proximity to the haul-out group from which mothers and pups caught.

It is surprising that BM did not father any of the pups as he and CM were both on the periphery of the capture area every year and CM fathered two of the pups. Both VM and CM fathered a pup caught in 1985 which was the first year that VM was seen in the study area and the second year that CM was seen in the study area. Interestingly, SS fathered a pup caught in the area two years before he was seen regularly on the periphery of this area. Until 1988, SS regularly hauled out in the adjacent study area. All males, except NE, were regularly seen in the study area in 1988 and yet none of them fathered the pups sampled in 1989.

There were seven pups whose fathers were not in the sampled males. The paternal bands in these pups (compared only between the pups whose

DNA was run on the same gel) were not shared and, therefore, it appeared that they were fathered by different males. This indicates that females giving birth to, and caring for, their young in the same area were mating with different males and would seem to indicate some mate choice. Lactating females frequent the Nursery area every year and because of the layout of the waterways, females must pass through several defended areas to reach the haul-out site. Therefore, they would come into contact with, and have the opportunity to assess, many different males.

The finding that so many of the pups had different fathers, suggests that females were mating with different males, and/or that females mate with more than one male. The results of this study (Chapter 2) suggest that the latter is possible. Both grey seal (Boness and James 1979) and elephant seal (Le Boeuf and Mesnick 1990, Campagna *et al.* 1993) females mate with more than one male in a breeding season and harbour seals appear to fit this pattern (Chapter 2). As grey seal and elephant seal females wean their pups and to return to the water, they must pass many males on the beach. These males can inflict serious, sometimes fatal, injuries on passing females while attempting to mate with them. It is possible that females will allow these males to copulate with them in an effort to avoid injury (Mesnick and Le Boeuf 1991). Although harbour seals mate in the water, where they are extremely mobile, the channels

in the Barachois are quite narrow, and, therefore, male harassment of passing females would be hard to avoid.

The finding that two mother-pup pairs had low band-sharing coefficients suggests that these females were fostering pups. Fostering behaviour, caring for young other than the biological offspring, has been documented in many species of birds and mammals (Reidman 1982, Emlen 1984), and also appears to occur in many of the phocid seal species (*e.g.* Burns *et al.* 1972, Reidman and Le Boeuf 1982, Boness 1990; also reviewed by Stirling 1975b, Reidman 1982 and Bowen 1991). In a behavioural study of harbour seals on Sable Island, ten percent of the females were found to foster for some part of the lactation period (Boness *et al.* 1992). Storms were responsible for separation of pairs and only females who had lost pups fostered. Lone pups have been found in the Barachois following storms or large-scale disturbances (ex. planes passing at low levels, boats discharging tourists on the haul-out locations, pers. obs.) and, therefore, it is possible that the circumstances causing fostering on Miquelon may be similar to that described for Sable Island.

CHAPTER 4

HARBOUR SEAL MATING STRATEGIES AT MIQUELON

The harbour seal mating system is still unknown, although most evidence suggests some degree of polygyny, which is common among mammals and prevalent among pinnipeds (Stirling 1983, Boness 1991, Le Boeuf 1991). As in most mammals, female harbour seals provide all the nutritional requirements for the young and, therefore, males are freed from parental care responsibilities. Also, like many other pinnipeds, female harbour seals gather in aggregations in predictable locations during the breeding season (*e.g.* Boulva and McLaren 1979, Krieber and Barrette 1984, Renouf and Lawson 1986, Davis and Renouf 1987, Thompson 1989), making it possible for males to compete for access to a number of females and increasing the potential for polygyny (Emlen and Oring 1977, Davies 1991). Unlike most phocid species, female harbour seals and their pups enter the water regularly after parturition and this movement would limit the ability of males to monopolize females directly (for a review of pinniped lactation strategies see Oftedal, Boness and Tedman 1987 and Bowen 1991).

Harbour seals usually mate in the water (Boulva and McLaren 1979, Allen 1985, Almon 1988), making copulation difficult to witness. Allen (1985)

described four copulations she observed over eight years. All of these copulations occurred either on land or in the shallows along the shoreline. Venables and Venables (1955, 1957) described three main components to mating behaviour: rolling, bubble-blowing and copulation. However, the sex of both members of the pairs could not be identified (Venables and Venables 1955). Because the timing of these behaviours appeared to be outside the mating period and are similar to those described for younger animals by other authors, it is likely that play was misinterpreted as mating behaviour (Thompson 1988). Because harbour seal mating is rarely witnessed, it is difficult to determine with whom individuals mate and, thus, the mating system (Boness *et al.* 1993).

Histological evidence indicates that harbour seals ovulate at or near the end of lactation (Fisher 1954, Bigg 1969, Boulva and McLaren 1979), which occurs approximately 23-24 days post-partum (Rosen 1990, Muelbert 1991). Males are in breeding condition at least one month beyond the time of pup weaning and, therefore, remain in breeding condition beyond the receptive period of females (Boulva and McLaren 1979). Individual females are remarkably consistent in their birthing dates between years (Temte 1991), and it appears that females at Miquelon are relatively synchronous in their oestrus as 95% of females pupped within a 15 day period (Rosen 1990). Clearly, if individual females are consistent in their annual birthing dates, and females

within a population are relatively synchronous in their oestrus, then female aggregations will be predictable in time. A combination of synchrony in oestrus plus female clumping suggests a low potential for polygyny in harbour seals and this suggestion is supported by the minimal sexual dimorphism observed in this species (McLaren 1993).

Haul-out behaviour of harbour seal males varies between locations during the breeding season. In locations where there is unlimited beach for hauling out, males are found scattered, some remaining alone and others hauling out in association with females and pups (Boulva and McLaren 1979). Some males show site fidelity within (Davis and Renouf 1987, Thompson *et al.* 1989), and between years (*e.g.* Thompson 1989). There are reports of "bachelor" male herds, which may include males that are driven off during inter-male competition (Knudtson 1977, Slater and Markowitz 1983, Kovacs *et al.* 1990). Thompson (1989) found that there was some degree of sex segregation at different haul-out sites. However, the sex-ratio at sites which had been used predominantly by males early in the breeding season changed to include more females, when females were presumably in oestrus. The changing of sex ratios in the haul-out groups at the time of mating, in combination with the presence of wounds on some of the males in the predominantly male herds, led him to speculate that these males may be involved in mating. Because most aggressive interactions and mating occur in the water, Thompson (1989)

concluded that it is not possible to assess the mating system of harbour seals based on the terrestrial distribution of this species during their breeding season. Instead, he argued that aquatic distributions and breeding status of males must be examined. In support of this argument, Thompson *et al.* (1989) found that the time spent ashore by some adult males was significantly greater in August, after the mating period, than it was immediately before and during the mating periods.

In harbour seals, females enter the water regularly during lactation (*e.g.* Perry and Renouf 1988) and mating, which follows lactation, occurs in the water. Therefore, establishment of aquatic territories may allow males greater access to females as they move to and from haul-out areas. Some authors have argued that maintaining a territory in the water would be very difficult for seals, as water represents a three-dimensional, boundaryless medium in which these animals have increased mobility (Bartholomew 1970, Stirling 1975a, Sullivan 1981). However, Weddel seals and Jaun Fernández fur seals establish aquatic territories (Francis and Boness 1991, Kaufman *et al.* 1975). There are also fish species which defend three-dimensional territories (*e.g.* Nursall 1977, Doherty 1983), although these may have landmarks which serve as boundaries.

Aggression among males becomes apparent near the end of lactation, as ovulation approaches (Davis and Renouf 1987, Thompson 1988). Males begin to show lacerations on the head, neck and tail regions, which has been

interpreted to represent intermale competition for mates (Sullivan 1981, Davis and Renouf 1987, Thompson 1988).

Sullivan (1981) suggested that agonistic aquatic interactions between male harbour seals, which occurred most frequently during the weaning and mating period, probably play a role in establishing dominance hierarchies. He speculated that male aquatic displays may allow receptive females to assess male quality and aid in their mate choice. Territorial males of most species engage in agonistic interactions within territories and use displays to delineate boundaries and advertise that territories are occupied. It is also possible that the agonistic encounters and displays of male harbour seals could be involved in territory establishment and maintenance.

The purpose of this study is to determine: 1) if male harbour seals are competing for females by displays and/or territorial maintenance and 2) whether these competitive tactics are linked to siring progeny, as determined in Chapter 3.

METHODS

Study Sites:

The seals gathered in six discrete groups on the sandbars at the beginning of the breeding season and frequented these sites until the end of lactation (Figure 4.1). After weaning, only the North Side and South Social are used for hauling out.

The Nursery site (observation location 1 on Figure 4.1) is used only by females, with or without pups, and occasionally by immatures. In this location two spots were used consistently. The South Social (observation location 2 on Figure 4.1) is regularly used as a haul-out site by approximately 150 seals of all age classes and both sexes until after weaning when the numbers increase to more than 250. The North Side groups include seals of all age classes, including mother-pup pairs, and some adult males. Counts of animals on the North Side range from 150 to 350 throughout the summer months. The final haul-out location is in the Goulet de Langlade (observation location 3 on Figure 4.1) where numbers can vary, depending on the amount of disturbance in the Barachois, but most commonly 5 - 18 animals haul-out.

Each May blinds were erected on the sandbars at all of these three haul-out sites (Nursery, South Social and Goulet) immediately prior to parturition. The blinds were placed approximately 5 metres from the water at low tide, and

as close to the haul-out location as possible without disrupting the haul-out. There has been no evidence that the seals are disturbed by the presence of the blinds over the 10 year period that blinds have been erected in the Barachois.

Blinds consisted of rectangular canvas covers with 0.6 by 0.6 metre vinyl windows on three sides and a zippered entry in the fourth side. These covers were placed over 1.5 metre high aluminum frames which were secured to one-square-metre bases mounted on four steel angle-iron legs, 1.5 metres long. Tire rims welded to the blind legs were buried in the sand to increase blind stability. Starting at one leg of the Nursery blind, metal stakes with surveyors tape tied to them were buried in the sand at 5 metre intervals for a visibility reference on foggy days.

Data Collection:

Preliminary work for this study started in the summers of 1985 and 1986.

The animals were observed for a total of 7 months in two consecutive breeding seasons (May - August, 1987 and May - July, 1988). Data included in this study were collected from 25 May to 09 August in 1987 and 28 May to 11 July in 1988 for a total of approximately 540 hours observation hours. A total of 52 hours of behaviour were recorded on video tape over the two seasons.

Data were collected daily, weather permitting, from the beginning of parturition to the end of weaning except in 1987 when data collection ended after the moult in August. Daily observations started approximately two hours before high tide, as determined by St. Pierre and Miquelon tide tables. Observation sessions continued until all adult males in the observation area had hauled out or ebb tide, whichever came last, except on those days when animals were caught for blood sampling purposes. On blood sampling days, observation sessions lasted until approximately 2 hours after low tide. During observation periods the seals' behaviours were recorded on data sheets and using a portable JVC GZ-X3 video camera with an 8-48 mm zoom lens and JVC videocassette recorder (model BR-1600U), all powered by a deep-discharge 12-volt battery. The following data were recorded:

1. At the beginning of each days' observations the time of day was recorded as well as meterological information including: time of high tide, wind direction, wind speed and visibility. Wind speed was recorded as either mild (no wind to slight breeze causing only ripples on the water), moderate (not mild nor strong) or strong (causing white-caps on the water). Stakes, with flagging tape attached, were placed at 5 metre intervals to measure visibility. These variables were recorded every hour

unless they changed before the end of an hour, in which case the changes were noted.

2. All adult male display behaviours were video taped including audio notes on the time of recording, number of seals in the area (hauled out and in the water), any events immediately preceding displays, location of the display relative to permanent land marks and response to displays by other nearby adult males. Taping commenced as soon as a display started, noting time that the display started and the number of slaps occurring before taping started, if necessary.

3. Interactions between adult seals (including agonistic encounters), noting distance of interaction (in adult seal lengths) from permanent landmarks, sex of actors, preceding events and consequences.

4. Details on the composition of haul-out groups including the total number of seals and, when possible, the sex and age class of individuals composing the group as well as identification of tagged animals.

5. Haul-out locations and times were noted for displaying males.

Locations of displays were recorded to determine whether individual males were displaying at consistent locations, as support for the hypothesis that males were defending particular areas. Locations of displays were noted relative to permanent landmarks (points of land, stakes hammered into the ground, floating buoys and painted rocks) and using these boundaries as limits, the total amount of shoreline associated with each male was measured. Shoreline was measured by pacing (one stride = approximately one metre) the distance between boundaries at low tide.

Video tapes were viewed upon return from the field site. Meteorological variables, display type and preceding incident, were coded. All displays were timed using a stop watch and the number of slaps within each display were counted, as a measure of display vigour. Times at which events occurred were converted to an integer scale relative to the time of high tide.

Analysis of variance (ANOVA) and correlations were used to analyse the data. Residuals were examined by plotting them against means (Draper and Smith 1981). If the plots of residuals against means were unacceptable (were not a random scatter of points around zero), a non-parametric Kruskal-Wallis test was used to verify ANOVA results. The Scheffé test was used for post-hoc comparisons of any significant ANOVA results involving more than two groups (criterion of $p < 0.05$). All analyses were performed using SPSS-X.

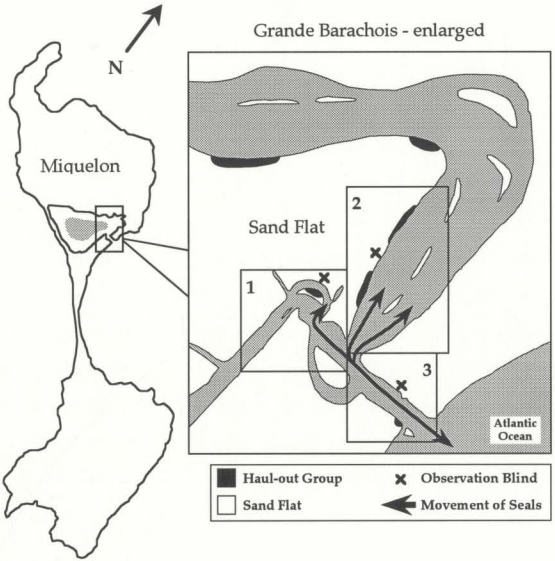

Figure 4.1: Sketch of complete study area within the Grande Barachois, indicating the three observation locations (**1** = Nursery, **2** = South Social and **3** = Goulet), haul-out locations, and direction of movement of seals into and out of the Barachois.

RESULTS

Reproductive Chronology:

The sequence of events over the breeding season in the Nursery area is represented in Figure 4.2. The first haul-out in the Nursery area (Study Site 1) occurred on 15 May in 1987, and on 19 May in 1988, although seals were hauling out in other locations within the Barachois before these dates. It was on these dates that the first pup was born in the Nursery area and the last births were recorded on 03 June in 1987 and 05 June in 1988. The period ending with the last births was considered the Pupping Period, which was followed by the Lactation Period. The first weaned pups in the Nursery area appeared on 16 June in both years and, therefore, this date was designated as the beginning of the Weaning Period. The last recorded haul-out in the Nursery area was on 30 June 1987 (included one mother-pup pair) and 07 July 1988 (weaners and a lone female).

Displays:

Displays were performed by males only (although a blind mother was regularly seen slapping the water with her foreflipper, in the shallows directly in front of her pup when the pup had not followed her off the beach). They involved slapping of the foreflippers (11%), hindflippers (2%), or a

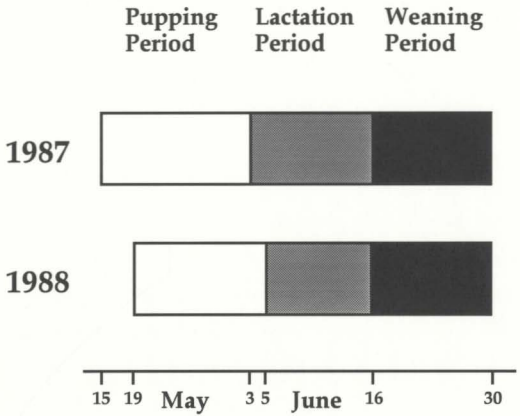

Figure 4.2: Reproductive chronology in the Nursery area in 1987 and 1988.

combination of both fore- and hindflippers (87%). Some displays were accompanied by growling, snorting and bubble blowing (24%), and others were accompanied by energetic head swinging back and forth while holding debris (such as sticks, algae and plastic bags) in the mouth (5%). Displays consisted of 1 - 30 slaps ($\bar{x}$ = 5.4, SE = 0.20, n = 435) and lasted from 1 sec to 5 minutes ($\bar{x}$ = 43.3 sec, SE = 2.45, n = 348). Longer displays had more slaps ($r = 0.7359$, $p < .01$, n = 348).

Harbour seal displays are extremely conspicuous. A loud sound is produced when the flippers slap the water and on a calm day, the sound travels great distances. On windy days the airborne sound can be masked by ambient noise but the wind tends to carry the water spray, resulting from displays, creating a dramatic plume of water above the displaying male.

A total of 450 displays were observed. Most displays (87%, n = 389) were by 9 adult males. One juvenile male displayed (1% of displays, n = 5). Unknown individuals accounted for 12% (n = 56) of the displays. Identified males (excluding the juvenile) displayed at significantly different rates ($F_{8,606} = 8.542$, $p < .001$).

The hourly rate of displaying ranged from 0.00 to 0.23 displays/hr ($\bar{x}$ = 0.01 displays/hr, SE = 0.00, n = 653), and was significantly different between time blocks relative to high tide. Display rate was much higher during the two hours immediately following high tide ($\bar{x}$ = 0.0138, SE = 0.002, n =

187 and $\bar{x}$ = 0.0139, SE = 0.002, n = 179, respectively) than during the third hour following high tide ($\bar{x}$ = 0.005, SE = 0.001, n = 140) ($F_{4,648}$ = 5.468, $p < .001$). No displays were recorded from 5 to 8 hours after high tide and, therefore, these blocks of time were excluded from further display analyses.

In both years, displays were already occurring in the Goulet (Study Site 3) when we arrived at the field site (12 May 1987 and 17 May 1988). Displays in the Nursery (Study Area 1) area began later. The first displays in the Nursery area occurred on 21 May 1987 and 19 May 1988. The last displays in the Nursery area were recorded on 03 July 1987 and 07 July 1988. These were also the last displays heard in the entire Barachois.

Displays occurred throughout the breeding season. The overall rate of displaying did not differ between the Pupping, Lactation, and Weaning Periods ($F_{2,662}$ = 2.138, $p > .05$).

Context of displays:

There was a positive correlation between display type and preceding incident, such that the more intense displays (those accompanied by growling, snorting, bubble-blowing or swinging an object) occurred most often following a fight (r_s = 0.246, $p < .01$, n = 450). Seven percent of the displays occurred following a fight and chase, and 23% of the displays occurred simultaneously

with another male displaying nearby. The preceding incident for the remaining 70% of the displays was not apparent. Displays following fights had significantly more slaps ($\bar{x}$ = 8.8, SE = 1.178, n = 30) than either simultaneous displays ($\bar{x}$ = 4.3, SE = 0.309, n = 106), or displays with no apparent preceding incident ($\bar{x}$ = 5.4, SE = 0.227, n = 299) ($F_{2,432}$ = 15.27, $p < .001$; X^2 = 21.67, $p < .001$). Displays following fights were also significantly longer ($\bar{x}$ = 84.2 sec, SE = 14.287, n = 228) than either simultaneous displays ($\bar{x}$ = 35.0 sec, SE = 4.447, n = 95), or those in which the preceding incident was unapparent ($\bar{x}$ = 42 3 sec, SE = 2.864, n = 228) ($F_{2,345}$ = 11.57, $p < .001$). However, the vigour (number of slaps in a display divided by the duration of the display) of displays did not vary significantly with the preceding incident ($F_{2,345}$ = 1.12, $p > .05$).

Displays were associated with all of the male-male (n = 27) aggressive interactions but not any of the 10 male-female encounters.

Display Locations:

On the basis of simultaneous display locations, display locations following fights and analyses of other display variables, it appeared that male harbour seals were establishing and maintaining territories. Displays were highly site-specific such that the Goulet males were never seen displaying in the Nursery area, nor were Nursery males ever seen in the Goulet area ($F_{8,380}$

= 993.58, $p < .001$). In addition, males in both the Nursery and Goulet displayed in specific locations within these areas (see Figure 4.3 and Table 4.1). Because few displays occurred in the South Social area over the two years of observation (n = 18) and 30 % were by a juvenile male, the South Social data were excluded from the following analyses and all comparisons were made between the Nursery and Goulet areas.

Ninety percent of the 355 displays by 9 known males were at specific areas referred to as grid lines (Figure 4.3). Simultaneous displays between adjacent males, and displays following fights, also occurred at these same grid lines (Table 4.2 and 4.3). The locations of displays established in 1987 were maintained by most males through the following year. The exceptions were when two new males (CF and SS) began displaying in the Nursery area, and one new male (GB) began displaying in the Goulet, in 1988. These additions affected the adjacent male's display locations in the Nursery but not the Goulet.

The rate of displaying increased with the number of grid lines at which males displayed ($F_{3,606} = 18.678$, $p < .001$). Males displaying at only one ($\bar{x}$ = 0.0036, SE = 0.0007, n = 294) grid line, displayed at a significantly lower rate than males displaying at either two ($\bar{x}$ = 0.0143, SE = 0.0024, n = 92), three ($\bar{x}$ = 0.0155, SE = 0.0019, n = 179), or four ($\bar{x}$ = 0.0161, SE = 0.0030, n = 45) grid lines. The residuals were not acceptable so this result

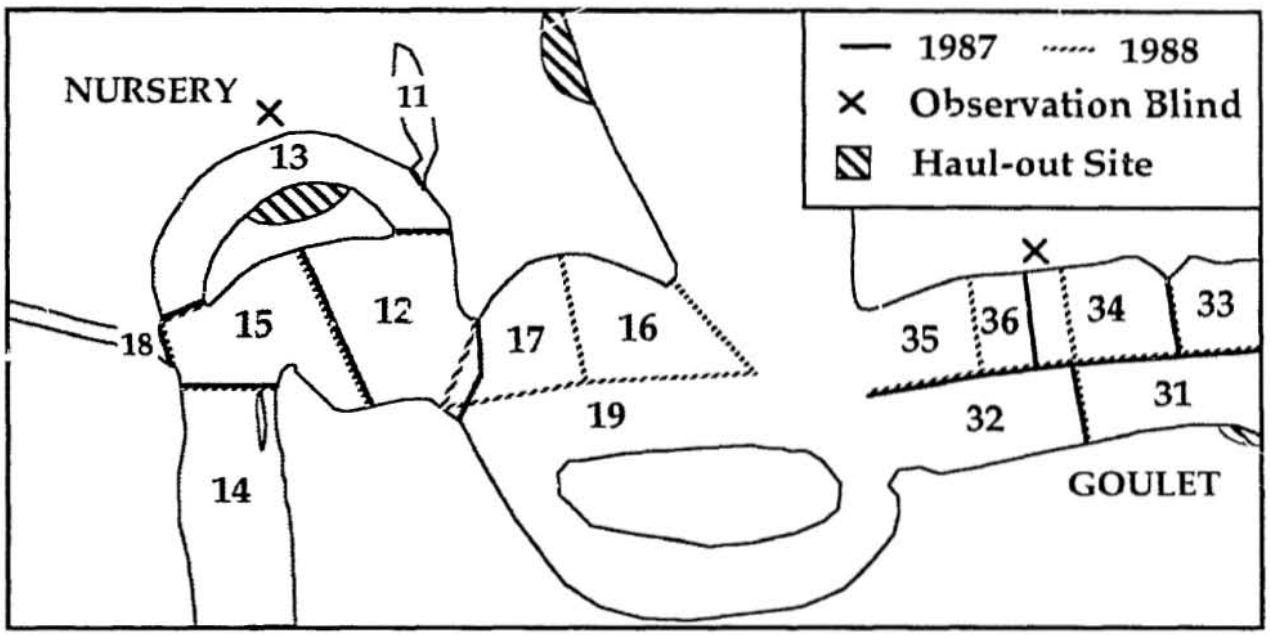

Figure 4.3: Sketch of Nursery and Goulet study areas with numbered grid locations for 1987 and 1988.

Table 4.1: Percent of displays performed by identified males in Nursery and Goulet grid locations.

		GRID LINES									
	ID	11	12	13	14	15	16	17	18	19	TOTAL (N)
NURSERY	CF		2				2	9		86	43
	VM	1	22	11	5	35		25		0.6	155
	BM					8			92		37
	CM				83	17					18
	SS						100				17
GOULET			32	33	34	35	36				
	PP			3	93		3				29
	NW				5	95					41
	SW		100								17
	GB				40	60					20

Table 4.2: Total number of simultaneous displays (number of displays in 1987/1988) between adjacent males at common grid lines.

LOCATION	MALE ID	CM	BM	CF	SS
NURSERY	VM	11 (2/9)	22 (21/1)	15 (0/15)	2 (0/2)
	CF				16 (0/16)
		GB	NW	SW	?*
GOULET	PP	11 (0/11)	3 (3/0)	2 (2/0)	8 (3/5)
	GB		4 (0/4)	2 (0/2)	
	NW			9 (6/3)	

* Unidentified Male

Table 4.3: Total number of displays (1987/1988), following fights and/or chases, occurring at the shared line between two grid areas.

LOCATION	MALE ID	COMMON GRID AREAS			
NURSERY		14/15	18/15	12/17	17/16
	VM	2 (2/0)	1 (1/0)	17 (6/11)	1 (0/1)
	CM	1 (1/0)			
GOULET		33/34	34/35	31/32	
	PP	2 (2/0)	1 (0/1)		
	NW		1 (1/0)		
	SW			1 (0/1)	

was checked with a non-parametric Kruskall-Wallis test and was found to be statistically significant ($X^2 = 81.71, p < .001$). When rate of display was divided by number of display locations, the rate of displaying was significantly higher for males displaying at two locations ($\bar{x} = 0.0072$, SE = 0.0012, n = 91) than for those displaying at only one ($\bar{x} = 0.0036$, SE = 0.0007, n = 294) ($F_{3,603} = 3.004, p < .05$). However, there was no significant difference between the hourly rate of display/boundary of males within study locations (Nursery: $F_{3,511} = 1.902, p > .05$; Goulet: $F_{1,90} = 1.672, p > .05$) (Table 4.4).

In 1987, two males (BM and CM) displayed at one grid line each, three males (PP, NW and SW) displayed at two gridlines each, and two males (VM and NW) displayed at three grid lines. In 1988, CF began displaying in the Nursery area, at two grid locations, adjacent to where VM regularly displayed. Both CF and VM had an additional display location in 1988 when SS started displaying late in the season.

Males differed in the number of locations in which they displayed, the amount of female movement between their display locations and the amount of shoreline contained between their display locations (see Table 4.5). In the Nursery area, VM displayed in the greatest number of locations and it was between these locations that females and pups hauled out.

Some displays occurred directly in front of an intruder male on the haul-out location (n = 12), at the grid line following a chase (n = 24), or both (n = 2). When

Table 4.4: Mean number of slaps in a display, duration of displays, vigour of those displays and display rate relative to the number of display locations at which males displayed in the Nursery and Goulet areas.

		NUMBER OF DISPLAY LOCATIONS				
		1	2	3	4	F
NURSERY	No. of slaps	3.6	6.5	6.2	5.8	6.23*
	Duration (sec)	35.1	47.9	56.5	53.4	1.97
	Vigour	0.38	0.19	0.29	0.16	5.38*
	Display Rate/hr	0.004	0.013	0.016	0.016	24.95*
	Hourly Rate/ Boundary	0.004	0.007	0.005	0.004	1.902
GOULET	No. of slaps	--	5.0	5.3	--	0.75
	Duration (sec)	--	30.5	26.9	--	0.27
	Vigour	--	0.36	0.29	--	1.24
	Display Rate/hr	--	0.016	0.013	--	0.18
	Hourly Rate/ Boundary	--	0.008	0.004	--	1.67

* $p < .05$

intruder males were discovered hauled out, displaying males displayed in front of the intruders and displays were vigorous, including snorting, bubble-blowing and growling. In most cases (n=10) these resulted in the intruder males moving toward the water and a fight followed in the shallows (see Aggressive Interactions below).

Male display locations in the Goulet were closer to each other than those in the Nursery, and there was less shoreline between display locations than in the Nursery (Table 4.5). In addition, no animals hauled out in the Goulet grids. The only Goulet location in which seals hauled out was too far from the blind to reliably identify displaying males.

Males in the Goulet displayed at a higher rate ($\bar{x}$ = 0.015 displays/hr, SE = 0.003, n = 94) than males in the Nursery ($\bar{x}$ = 0.009 displays/hr, SE = 0.007, n = 521) ($F_{1,613} = 6.07$, $p < .05$). The rate of displaying per grid line was not different between the two areas ($F_{1,605} = 2.011$, $p > .05$) and, therefore, the difference between rates of display between the Nursery and Goulet areas was attributable to the differences in numbers of display locations for males within each area.

Males in the Nursery area differed significantly in the number of slaps per display and the vigour of the displays (Table 4.4), when categorized by the number of locations at which they displayed. In general, number of displays, slaps/display and duration of displays all tended to increase with number of

Table 4.5: The number of display locations, approximate metres of shoreline contained between locations, and whether or not females would be encountered between display locations of each displaying male harbour seal.

LOCATION	MALE ID	NO. OF LOCATIONS	SHORELINE (metres)	FEMALES ENCOUNTERED		PUPS SIRED
				ON LAND	IN WATER	
Nursery	CF	2/3'	1060	No	Yes	0
	VM	3/4'	2045	Yes	Yes	1
	BM	1	55	No	No	0
	CM	1	>200	No	Rare	2
	SS	2	90	No	Yes	1
Goulet	PP	3	121	No	Yes	--
	NW	2	88	No	Yes	--
	SW	2	134	No	Rare	--
	GB	3	40	No	Yes	--

' SS started displaying late in 1988, adjacent to VM's and CF's display locations, thereby increasing the number of locations in which these males displayed.

locations while vigour (number of slaps in a display divided by the duration of that display) tended to decrease. No differences were found among the Goulet males.

Aggressive Interactions:

All aggressive interactions involved growling, snorting, biting or lunging, and either physical contact or chasing. A total of 37 aggressive interactions were observed over the two years. Twenty-seven were known to be between males and 10 were between males and females. The aggressive encounters between males occurred throughout the breeding season whereas the aggressive interactions between females and males started in the Weaning Period (from 22 June - 30 June, 1987 and 20 June - 5 July, 1988). All male-male aggressive encounters had displays associated with them while those between males and females did not.

Fights between males, on land or in the shallows (n = 12), rarely resulted in head or neck wounds. In all cases, the displaying male tried to grab the unidentified male's hindflippers and the intruder spent a majority of time spinning to face the displaying male, thereby avoiding being grabbed by the flippers. Open bleeding wounds could be seen on the hindflippers of unidentified males, and toward the end of weaning, it became more difficult to find uninjured sections of skin on the hindflippers of males during blood sampling. In only one case did a

displaying male inflict neck wounds on the intruder. In this encounter the intruder was able to keep his hindflippers away from the displaying male and the displaying male started to lunge at the intruder's neck, inflicting large wounds at the side of the neck. In all of these male-male encounters the intruding male eventually entered the water and quickly left the area, with the displaying male pursuing as far as the grid line where he displayed.

Male-female aggressive interactions (n = 10) began in the Weaning Period in each year. In 1987 the first aggressive interaction occurred on 22 June and in 1988 on 20 June. Only two of the encounters occurred in the Goulet while the remainder occurred in the Nursery. In all cases, a male tried to mount a female either on the sand or in the shallows, and the female growled and snorted while biting the male's neck. These bites always resulted in open bleeding wounds. In no case did a male inflict wounds on the female and, in most cases, the encounters were of short duration, with the females managing to escape the males. In only one encounter was copulation suspected as the female lay docile for 12 minutes with the male on top of her. Certain copulation was never witnessed.

Male-male fights occurred during male-female encounters in the Goulet, but never in the Nursery. As a male would try to mount the female, other males would approach and try to remove the mounting male.

Haul-out Behaviour:

The haul-out of animals in the Nursery area was composed primarily of adult females and pups and occasionally juvenile animals (Figure 4.4). Adult males, other than the displaying male, were rarely seen in this haul-out group (see Aggressive Interactions above). In the Goulet area, the haul-out consisted mainly of males, including juvenile and adults. I saw females in this group on only two occasions and they did not remain longer than 8 minutes, possibly as a result of the harassment by males (see Aggressive Interactions above).

The number of animals hauled out was significantly different between hour blocks around high tide. Significantly more animals were in the haul-out groups during the third ($\bar{x}$ = 12.60, SE = 133, n = 146), fourth ($\bar{x}$ = 13.37, SE = 1.87, n = 106) and fifth hour ($\bar{x}$ = 18.60, SE = 4.29, n = 54) following high tide, when the sandflats were exposed, than during the hour immediately before high tide ($\bar{x}$ = 3.89, SE = 1.11, n = 156) when the sandflats were still covered by water, based on a parametric test ($F_{10,889} = 6.42$, $p < .001$) and checked by a non-parametric test ($X^2 = 100.85$, $p < .001$). The number of animals hauled out correlated with hour relative to high tide (r = 0.2051, p = <.01, n = 939), wind speed (r = -.1375, $p < .01$, n = 440) and visibility (r = -.1551, $p < .01$, n = 440) but not with wind direction (r = 0.0088, $p > .05$, n = 440). The mean number of animals hauling out in the Nursery and Goulet areas was significantly different between the three stages of breeding (Pupping, $\bar{x}$ = 16.54,

SE = 1.77, n = 183; Lactation, $\bar{x}$ = 21.88, SE = 2.08, n = 119; and Weaning, $\bar{x}$ = 5.06, SE = 0.03, n = 598). More animals hauled out in the Lactation Period than either the Pupping or Weaning Periods, and more animals hauled out in the Pupping Period than in the Weaning Period, based on a parametric ANOVA ($F_{2,897} = 71.43$, $p < .001$), checked by a non-parametric Kruskal-Wallis ($X^2 = 9.32$, $p < .01$).

Haul-out of Displaying Males:

The amount of time that displaying males spent hauled out was negatively correlated with their display rate (r = -.188, $p < .01$, n = 655) and positively correlated with the number of animals in the haul-out (r = 0.277, $p < .01$, n = 655). Males spent more time hauled-out during the third and fourth hour after high tide ($\bar{x}$ = 16.55 min/observation hour, SE = 2.202, n = 132, and $\bar{x}$ = 21.34 min/obs. hr, SE = 3.309, n = 71, respectively) than during the hour preceding high tide ($\bar{x}$ = 1.69 min/obs. hr, SE = 1.167, n = 71) and the two hours immediately following high tide ($\bar{x}$ = 3.11 min/obs. hr, SE = 0.9113, n = 169, and $\bar{x}$ = 9.28 min/obs. hr, SE = 1.521, n = 165, respectively) ($F_{4,003} = 18.07$, $p < .001$; $X^2 = 93.99$, $p < .001$).

Display rate did not correlate with the number of animals hauled out (r = -.061, $p > .05$, n = 665). Males displaying at only one grid line spent significantly more time hauled-out ($\bar{x}$ = 18.62 min/obs. hr, SE = 1.505, n =

314) than any other males ($F_{3,637} = 28.19$, $p < .001$; $X^2 = 57.70$, $p < .001$) and males in the Nursery area spent significantly more time hauled-out than the males in the Goulet ($\bar{x}$ = 12.06 min/obs. hr, SE = 0.968, n = 552, and $\bar{x}$ = 3.73 min/obs. hr, SE = 1.283, n = 103, respectively) ($F_{1,653} = 13.02$, $p < .001$; $X^2 = 11.09$, $p < .001$).

Although the rate of displaying did not differ between breeding periods, males spent more time hauled-out during the Lactation Period ($\bar{x}$ = 16.31 min/obs. hr, SE = 2.035, n = 87) than they did during the Weaning Period ($\bar{x}$ = 9.31 min/obs. hr, SE = 0.979, n = 441) ($F_{2,652} = 4.05$, $p < .05$; $X^2 = 9.32$, $p < .01$).

When males were not hauled out and not displaying, it was not uncommon to see them floating in one location, near a grid line, for extended periods. On calm days, when males were not visible, it was possible to see bubbles erupting at the water's surface in these locations. These males also appeared to patrol the water between their display sites. VM spent a small proportion of each day swimming downwind of the haul-out group between his boundaries and would occasionally approach lone females in the haul-out group. Generally, females would move away from him, snorting.

Displaying males that did haul out clearly lost weight over the breeding season and, although it is not possible to say that they never left the area, it would seem that they did not feed regularly. Capelin entered the Barachois each

year, and when they did, most seals appeared to feed on them, including the displaying males.

Paternities:

A total of eleven mother-pup pairs, caught in the Nursery area, and five adult males (four displaying males from the Nursery area and one non-displaying male from an area outside of the study areas) were included in the paternity analysis (Chapter 3). Three of the displaying males had fathered pups (VM, CM, SS) while one displaying male (BM) and the non-displaying male had not (see Figure 4.5 and Table 4.5). CM fathered two of the pups sampled during the years in which he was displaying in the Nursery area. VM fathered one pup caught in the Nursery area in 1985, which was the year VM started displaying in grid areas 12, 13, and 15, of the Nursery site. In the previous year, VM displayed adjacent to these locations, in grid area 17. SS fathered one pup caught in the Nursery area in 1986, which means that the pup was conceived three years before he started displaying in the Nursery site. In these years, he regularly hauled out in the South Social group.

BM displayed at a significantly lower rate ($\bar{x}$ = 0.005 displays/hr, SE = 0.001, n = 131) than the other males ($\bar{x}$ = 0.01 displays/hr, SE = 0.001, n = 307) ($F_{1,436} = 10.807$, $p < .01$). However, BM's display vigour (number of slaps

in a display divided by the duration of the display) was not significantly different from that of the other males ($F_{1,185} = 0.009$, $p > .05$).

Amount of time spent hauled out differed significantly between males who had fathered pups and the one who did not (BM) ($F_{1,463} = 5.232$, $p < .05$), with BM spending more time hauled out ($\bar{x} = 18.2$ min/obs. hr, SE = 2.23, n = 140) than the males that had sired pups ($\bar{x} = 12.6$ min/obs. hr, SE = 1.27, n = 325).

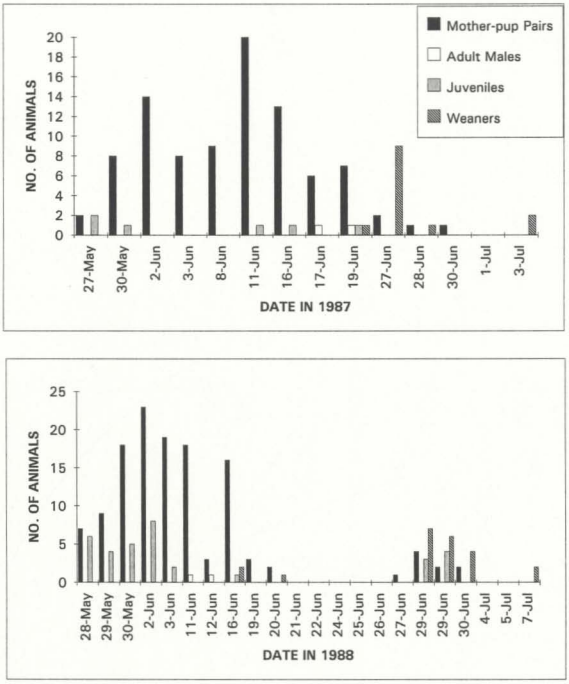

Figure 4.4 : Daily numbers of animals hauled out in the Nursery area over the breeding season.

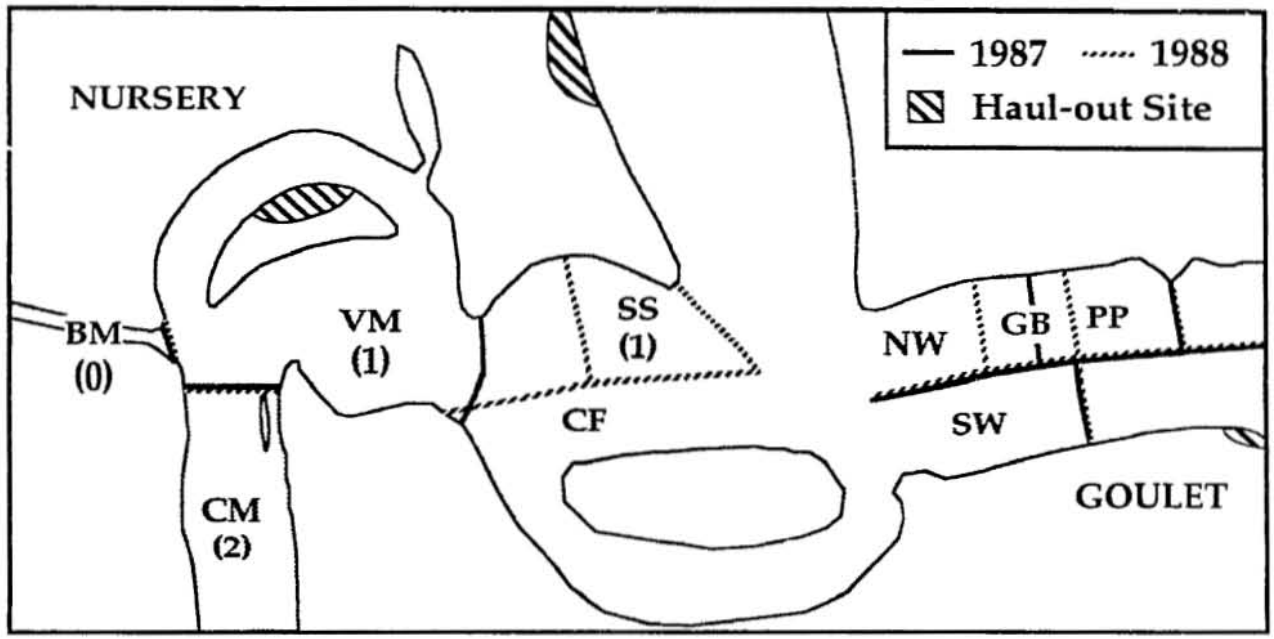

Figure 4.5: Territory boundaries of identified males (with number of pups sired) in the Nursery and Goulet study areas in 1987 and 1988.

DISCUSSION

The results of this study indicate that the haul-out behaviour of harbour seals during their breeding season at Miquelon was similar to that reported for this species during the breeding season in other locations. Given this similarity, the behaviour of males at Miquelon is strikingly different from that described for males in other locations.

Haul-out:

The number of animals hauled-out was significantly correlated with time relative to high tide, which is not surprising as the sandbanks on which these seals haul out are completely covered at high tide. The sandbanks are not completely exposed until approximately two hours following high tide, and it was in the third hour following high tide that the greatest number of animals were hauled out. Terhune and Almon (1983) also found that seals did not haul out at high tide, despite haul-out space being available.

Wind speed, but not direction, was negatively correlated with the number of animals hauled out, as was visibility. Strong winds caused rough sea-states and other studies have found that both of these meteorological variables have an affect on the size of harbour seal haul-out groups (Boulva and McLaren 1979, Schneider and Payne 1983, Kovacs *et al.* 1990).

To date, the effect of visibility has not been reported as a factor influencing the number of animals hauled out. This is most likely because many studies involving seal censusing are conducted from a distance (*e.g.* Terhune and Almon 1983), and fog would inhibit the ability to see and count those animals. It is unlikely that reduced visibility decreased my counts because the Nursery area observation blind was situated no more than 8 - 10 metres from the animals. In addition, minimum visibility in this study was 50% which meant that the haul-out site was always visible. On foggy days, when vision was already limited, winds were usually low, making sounds clearer. On these sorts of days, the least bit of disturbance cause the seals to race for the water. The effects of disturbance on the number of seals hauling out in the breeding and non-breeding seasons has been documented in several locations (Renouf *et al.* 1981, Schneider and Payne 1983, Allen *et al.* 1984).

The haul-out pattern of harbour seals at Miquelon during the breeding seasons was similar to that described by authors for other populations of this species. The Nursery group was composed primarily of mother-pup pairs, the Goulet group was male-dominated and in the South Social area there was a group composed of both sexes and all ages. The number of animals hauling out in the Nursery and Goulet areas was significantly greater during the Pupping and Lactation Periods than during the Weaning Period, which suggests that some sites are used preferentially during these stages of the breeding cycle. This finding has

been reported at Miquelon in previous years as well (*e.g.* Lawson and Renouf 1985, Davis and Renouf 1987). Females appear to use specific locations for hauling out during these periods in Orkney (Thompson 1989), Shetland (Venables and Venable 1955), along the coasts of Nova Scotia (Boulva and McLaren 1979), New Brunswick (Kovacs *et al.* 1990) and California (Allen, Ribic and Kjelmyr 1988), but not at Sable Island (Godsell 1988) where haul-out space is always available.

Several authors have suggested that female segregation during the pupping and lactation periods may reflect their use of haul-out locations which are more sheltered or more readily accessible through tidal fluctuations (Venables and Venables 1955, Boulva and McLaren 1979). This factor may account for the finding that there is no apparent segregation on Sable Island where there is unlimited homogeneous haul-out space available (Godsell 1988). It is also possible that segregation of females with pups may reduce the amount of disturbance by other seals during the lactation period (Allen *et al.* 1988, Thompson 1989), as females with pups are known to drive other seals away (Lawson and Renouf 1985).

By gathering together in nursery aggregations, individual female harbour seals would benefit from being able to spend less energy scanning for predators or sources of disturbance. As harbour seal group size increases, individual scan time decreases while overall vigilance is increased (Kreiber and Barrette 1984,

Terhune 1985, Da Silva and Terhune 1988). Females with pups spend more time in general scanning (scanning both water and land) than any other seals in a mixed haul-out group (Renouf and Lawson 1986). Perhaps when females gather into isolated groups to care for their young, they may benefit by the overall increased vigilance afforded by other mothers.

The Goulet haul-out group was almost exclusively composed of males, of varying age classes, as indicated by size and degree of scarring on individuals. Exclusive male and male-dominated haul-out groups have been reported in Orkney (Thompson 1989), New Brunswick (Kovacs *et al.* 1990), and California (Knudtson 1977, Slater and Markowitz 1983, Allen *et al.* 1988) but not Sable Island (Godsell 1988). There is some controversy over the reason for male-dominated haul-out groups during the breeding season (see below).

Displays:

The displays described in this study were very similar to those described by Sullivan (1981), including the display-type variation. In this study, displays involving both the fore- and hindflippers were most frequent, followed by foreflipper-only and then hindflipper-only displays. Sullivan found that hindflipper-only ('lobtailing') was the most common form of display, followed by foreflipper-only and then a combination of fore- and hindflipper. Occasionally, males in this

study picked up debris in their mouths and swung it in the air, which was described by Sullivan as mouthing or manipulating sea palm or floating surfgrass.

Sullivan (1981) reported longer displays on average than those reported here (4.9 minutes compared to 43 seconds), although they did fall within my range of up to five minutes. The duration of displays by captive harbour seal males (Almon 1988) were similar to those reported in this study.

Male harbour seals started displaying immediately before parturition and continued until the end of weaning. There were no displays by identified males after all pups had been weaned in 1987 (in which year observations continued through August). Displays by captive male harbour seals also abruptly ended following mating (Almon 1988). Thus, it appears that male displays are associated with the breeding season only and relate to mating. Sullivan (1981) found that males displayed throughout the year although the frequency of displays was much lower before and after the breeding season. He did not report the age classes of displaying animals, and it is possible that the displays he witnessed outside of the breeding season were by young animals playing rather than by adult males. In the present study, one juvenile male (3 years of age) displayed after the breeding season (July 7). Although Venables and Venables (1955) also described rolling and splashing behaviour (displays?), which they felt was associated with mating, Thompson (1989) argued that these behaviours were occurring outside of the breeding season and were probably play between immature animals. It is

most likely that adult male displays are limited to the breeding season and are exclusively related to mating.

Most displays result in plumes of water in the air above the displayer. Displays involving both fore- and hindflippers result in a series of at least two slaps, which on a calm day, sound much like gunshots and can be heard throughout the Barachois. The sounds produced by displays also carry underwater. Thus, these conspicuous displays should be easily detectable by neighbouring males (Wiley 1983) and whether or not males are submerged, they should always be aware of each others' displays.

Because sound travels about five times faster through water than air (Popper and Coombs 1980), it is possible that males could determine the location of the displaying male, by comparing the difference in arrival time between airborne and underwater signals (see Renouf 1991 for a review of seal hearing), in addition to other cues used by binaural animals in locating sound sources (see Mills 1972). This might explain how intruder males manage to enter territories and haul out, undetected. It may be possible for intruders to determine when they can cross a particular boundary unhindered by localising the display sounds produced by a territorial male at a distant boundary.

The displays of adult male harbour seals are ritualised, stereotyped threat signals. Ritualisation of threat signals, is thought to reduce the ambiguity of these signals. However, it also decreases the amount of information transmitted (Harper

1991). The receiver cannot be certain of the signaller's internal state or intentions concerning escalation of the interaction, based on ritualised signals (Caryl 1979, 1982) and, therefore, ambiguity may still exist. Interestingly, threat displays of several species of birds are poor predictors of attack (Caryl 1979). When male harbour seals discovered intruder males in the haul-out, they displayed directly in front of the intruders. In these cases, there was always a fight between the males. There were also chases without physical combat which suggests that although displays are clearly signalling territory ownership, some intruders tested signallers' intentions to defend those territories, by intruding.

Variation in display intensity may serve to signal to receivers in different locations or could serve to indicate different levels of aggression (Tinbergen 1959). The displays observed in this study appeared to vary in their intensity. Less intense displays (fore-, hindflipper or both) were more frequent than more intense displays (those accompanied by growling, snorting, bubble-blowing or swinging an object) which occurred most often in association with aggressive encounters between males. The cost of threat displays increases with their effectiveness in repelling competitors, because those which are most likely to cause a competitor to flee are also more likely to cause a fight, depending on the response of the recipient (Enquist *et al.* 1985). The increased risk of initiating a fight might explain why intense harbour seal displays were rarer than less intense displays.

Both this study and Sullivan's (1981) report simultaneous displays between males. In this study, these displays occurred consistently at shared grid lines. Although these simultaneous displays are much more dramatic, they are not unlike those been male Australian fur seals (*Arctocephalis fosteri*) and Steller sea lions (*Eumetopias jubatus*), which also engage in simultaneous threat displays at territorial boundaries (Gentry 1975, Miller 1975, Sandegren 1975).

Territories:

The results of this study suggest that adult male harbour seals defend aquatic territories. These territories are established and maintained through inter-male aggression and aquatic displays at territory boundaries. Displays were extremely site-specific, and it was possible to determine boundaries on the basis of display locations of identified males. These boundaries were confirmed through simultaneous displays at common grid lines between neighbouring males, as well as displays which occurred following chases to these same lines. The locations of territory boundaries were consistent between years except in three cases where new males established territories in the areas.

The Nursery area had three clearly defined territories in 1987 (Figure 4.5). Two of these territories had only one boundary (BM's and CM's), while VM shared a boundary with BM and CM, and had an additional boundary to the east. In 1988, two new males (CF and SS) established territories to the east of VM's

territory. When SS started to establish his territory late in 1988, he encroached on both VM's and CF's territories, causing VM's east boundary to shift, and creating a boundary between CF and SS.

The Goulet is a wide channel through which all seals, including females, must pass to enter and exit the Barachois. Male territories divided the channel longitudinally and into sections, such that each territory was along a stretch of shoreline. In 1987 there were three males who could be identified and for whom boundaries could be mapped (PP, NW, and SW). In 1988 a new male (GB) established a territory between PP and NW. This reduced the size of PP and NW's territories through the adjustment of boundary locations, but did not affect the number of boundaries at which they displayed.

There were few displays in the South Social area. The haul-out group there was composed of mixed sexes and age classes. As a result of the lack of displays there were no apparent territories in this area. The only observed displays were performed by a three year old juvenile male, sexed by the tag placement and aged by his tag colour. These displays occurred after females had weaned their pups and, therefore, after the breeding season.

In birds, males generally display during the breeding season to either attract mates or keep intruders from territories, or both. Increased rates of displaying in some species have been directly related to greater mating success (Vehrencamp, Bradury and Gibson 1989; Pruett-Jones and Pruett-Jones 1990), and experimental

manipulations of singing (surgical mutings) have demonstrated the significance of displays in acquiring and maintaining territories (Smith 1976, 1979, McDonald 1989). The rate of aquatic displaying by male harbour seals was highest at the time that there would be the greatest movement of seals through the water (in the two hours following peak tide), and there were no displays following the Weaning Period, suggesting that the display was directly related to female movement, as has been documented in sage grouse (Gibson *et al.* 1991). However, the rate of display was related to the number of boundaries at which males displayed and, thus, the number of neighbouring territorial males. This suggests that the primary function of displays is to maintain boundaries.

The song rate of territorial red-winged blackbirds does not vary with the number of neighbouring males, nor their proximity, but does decline as nest initiations decline, suggesting that the song is used to attract females (Shutler and Weatherhead 1991). However, males with higher song rates did not attract more females and, therefore, Shutler and Weatherhead (1991) concluded that the song functions primarily to announce that a territory is occupied. Unfortunately, they did not examine the size of song repertoires, which is known to affect both the number of copulations by males and the number of intrusions on territories in many passerine birds (Catchpole, Dittami and Leisler 1984; Searcy 1984; Yasukawa and Searcy 1985; Baker *et al.* 1987). The increased rate of displaying by male harbour seals when there is increased movement of seals through the

water suggests that territorial males were attempting to prevent intruder males from entering the territories at the time when intruders might be able to "sneak" across boundaries, hidden by the movement of females and pups.

Aggressive interactions witnessed during this study support the suggestion that males are being excluded from some locations. In every case in which intruder males were discovered hauled out with the Nursery group, the resident male forced them to leave the area through fights and chases to boundaries. It is not surprising that territorial males always succeeded in forcing hauled out intruders to leave territories. Intruders usually defer to territory holders (*e.g.* Krebs 1977, 1982; Davies 1978; Harvey and Corbet 1986; Gribbin and Thompson 1991).

All aggressive interactions observed in this study were between territorial males and intruders, as opposed to neighbours. The encounters varied in intensity with the most intense, or severe, physical fighting being between territorial males and hauled-out intruders. This suggests that greater effort is required to evict intruders as the length of time the intruder is allowed to remain within the territory increases. Great tits (*Parus major*) generally sing at their territory boundaries when they encounter established neighbours. However, when they encounter a new neighbour the interaction often involves physical contact as well as visual displays. The intensity of interaction increases when an intruder is found within

the territories and the degree to which the interactions increases is a function of the time that the intruder is allowed to stay in the territory (Krebs 1982).

More than half of the displays observed in this study were simultaneous displays between neighbouring males but no aggressive interactions (physical fights) were witnessed between them. The lack of aggressive encounters between neighbours may be as a result of the "dear enemy" (Fisher 1954) phenomenon functioning to prevent escalation of contests between neighbours. Once territory boundaries have been established, neighbouring territorial males are of little threat to each other. Therefore, there would not be a need to respond aggressively to a neighbour's display (Jaeger 1981). Because aggressive encounters can be costly, it is of benefit to territorial males to recognise neighbours. Most of the males in this study held their territories over both consecutive breeding seasons and three of the males in the Nursery area (BM, CM, and VM) held the same territories for four consecutive years (1985 - 1988). This would give ample opportunity for the males to become "familiar" with their neighbours, possibly recognising them on the basis of their displays, and could account for the lack of escalated aggressive encounters (fights) between territorial males.

Given the small sample sizes of pups and males, it is remarkable that any fathers were identified. Most notably, only two pups from 1985 were included in the analysis and both fathers were found in the males sampled. The paucity

of data makes it even clearer that territorial males are accruing some benefits (successful copulations) from holding territories. Through exclusion of males from territories, it would seem that females could benefit from hauling out within a territory in which they can care for their young undisturbed. Thus, sexual selection could favour territorial behaviour in this species.

Because harbour seals copulate in the water (Allen 1985, Almon 1988, Chapter 2), it is impossible to determine if seals are copulating within their territories. Territorial males in this study spent a majority of their time in the water and there were many periods in which males could not be located. In addition, territorial males spent even less time hauled out during the Weaning Period when mating is expected to occur. The possibility that males may have been following females from the Barachois to mate, cannot be ruled out as females are known to spend more time at sea (presumably to feed but possibly to mate) following lactation (Thompson *et al.* 1989).

There are three pieces of evidence suggesting that territorial males are not leaving their territories, even during the Weaning Period. First, although the amount of time that territorial males spent hauled out was less (on average, 7 min/obs. hr. less) during the Weaning Period than the Lactation Period, the rate of displaying did not differ among the three phases of the breeding seasons. It does not seem likely that males could leave their territories to copulate with females, without reduction in the display rate. Secondly, the high level of male

aggression within territories would make it even more unlikely that males could leave their territories with females, and pass through all other territories unhindered. Thirdly, the condition of males deteriorated over the season, judging from the apparent loss of mass, suggesting that males were expending a significant amount of energy on territorial defence and not replenishing stores by feeding. There is little food available within the limits of the Barachois and, therefore, seals would have to leave the Barachois to feed. If males were leaving their territories to feed then I would predict that they would not suffer such observable mass losses. It is clear that males will feed during the breeding season (also reported by Reilly and Fedak (1991)) as each year seals were observed feeding on capelin, which entered the Barachois annually to spawn. Territorial males were only observed to feed when the capelin entered their territories. Although these pieces of evidence are circumstantial they do not support the suggestion that males leave their territories even to mate. Thompson *et al.* (1989) studied the movements of five adult male harbour seals during the summer months, using radio-telemetry. These authors found that males varied in the amount of time they spent within and outside of the study area. Unfortunately, their data include all summer months and are not restricted to the breeding season, making comparison difficult.

Mating System:

To date, the mating system of harbour seals has remained undetermined. The phylogenetic and ecological constraints on this species, plus anecdotal observations of male behaviour during the breeding season, have lead most researchers to suggest that harbour seals should be mildly polygynous (Bigg 1969, Sullivan 1981, Stirling 1983). The results of this study indicate that some male harbour seals at Miquelon are defending aquatic territories. However, the territories do not appear to fit either those described for highly polygynous species (as in resource defence polygyny) nor those found on a classic lek arena.

The displays were site-specific for each male and occurred at territory boundaries, with neighbouring males performing simultaneous displays where boundaries were shared. The only time that displays did not occur at territory boundaries was when intruder males were discovered within territories. At these times, displays and fights were used to aggressively exclude intruder males from their territories. These pieces of evidence indicate that males were defending territories but it is not clear what resource exists to be defended. It is possible that the access routes to haul-out areas are the defended resource.

The paternity results presented here (Chapter 3) indicate that there is some advantage to holding a territory although the level of polygyny appears to be low. Further, the time at which male harbour seals begin displaying in territories is suggestive of a low level of polygyny. Males at Miquelon established territories

at the same time that females were gathering for birthing rather than before the females arrived. In highly polygynous species, in which males are defending either females or resources essential to females, males gather well in advance of the females to establish territories (Boness 1991). Early establishment of territories in these species is possible because males can predict where the females will aggregate for parturition. When females are widely dispersed, resources are patchy, or females are clumped but the cost of defending them is too high, then a lek system is likely to exist (Bradury 1981).

Female harbour seals do gather in predictable locations for parturition, and they are more synchronous in oestrus than many of the highly polygynous seal species. Ninety-five percent of females at Miquelon pup within a 15 day period (Rosen 1990) which would make oestrus much more synchronous than, for example, southern elephant seals in which copulation continues for more than one month (Campagna, Lewis and Baldi 1993). Such a synchronous oestrus, in combination with the tide-related movements of female harbour seals, would make it difficult for males to monopolize females directly. Further, females provide complete care for their young through lactation and, therefore, do not appear to require anything beyond an undisturbed haul-out space on which to nurse their young. Therefore, there is no immediate resource for males to defend, although females could indirectly benefit from territorial behaviour by having males excluded from the areas in which they care for their young.

Generally, a territorial male's reproductive success is thought to reflect the quality of his breeding territory, while reproductive success of lekking males is thought to reflect their self-advertising/display abilities and display location (Emlen and Oring 1977, Balmford *et al.* 1992). The territories on a lek contain no resources and males expend large amounts of energy in self-advertisement through visual, auditory or olfactory displays (Vehrencamp *et al.* 1989, Davies 1991). Lekking systems vary within and between species (Clutton-Brock *et al.* 1988, Pruett-Jones 1988, Apollonio *et al.* 1992), and it has been shown that lek territories differ in their quality (usually location within an arena), as measured by the relative number of matings occurring in each, as males compete for the better located territories (Emlen 1976, Apollonio *et al.* 1989a, 1989b, 1990, Festa-Bianchet *et al.* 1990). Many lekking species do not behave in a manner typical of a classic lek but rather appear to have behaviours intermediate between territoriality and the classic lek (Bradbury 1981). The behaviour of male harbour seals at Miquelon appears to fits onto a continuum between lekking and territoriality. The territories occur on routes taken by females to and from haul-out sites and, therefore, the males appear to be defending access routes and haul-out locations much like male Weddell seals.

Unlike highly polygynous species, male harbour seals establish their territories at about the same time that females arrive in the Barachois for parturition. Unlike classic lek systems, in which male display arenas are removed

from the areas frequented by females (Bradbury 1981), harbour seal territories are in the immediate vicinity of pupping sites. Male harbour seals are extremely aggressive when they encounter intruder males within their territory limits, in contrast to the expected emphasis on self-display rather than aggression. Most of these characteristics are common to those of California sea lion and Hooker's sea lion males, both of which are described as intermediate lekking species (Boness 1991), and some ungulate species (Clutton-Brock *et al.* 1988, Festa-Bianchet *et al.* 1990).

At Miquelon, seals begin to haul-out on sandflats as the tide falls, with the greatest number of animals present consistently during the third and fourth hours following peak high tide. Therefore, seals are moving to the haul-out sites in the two hours immediately following high tide, when the rate of displaying was highest. Thus, displays that function primarily to maintain territories would also be witnessed by females as they passed displaying males on their way to haul-out sites. In some species which establish terrestrial leks, females often visit several males before copulating with one, and it is possible that females assess male quality based on the displays or the outcome of intra-sexual competition (Emlen and Oring 1977, Payne 1984, Kirkpatrick and Ryan 1991, Gibson *et al.* 1991). Female harbour seals at Miquelon would necessarily pass many males daily and have ample opportunity to compare male display qualities. In addition, females could assess male quality on the basis of intermale aggression as has been

suggested for elephant seals and grey seals (Cox and Le Boeuf 1977; Boness, Anderson and Cox 1982), two species that are highly polygynous.

Several authors have suggested that male-dominated ("bachelor") haul-out groups may exist during the breeding season as a result of intermale competition. Subordinate males may be excluded from pupping locations (Knudtson 1977, Slater and Markowitz 1983, Kovacs *et al.* 1990). Thompson (1989) argued that some males in male-dominated groups had fresh wounds and scarring on their head and neck regions, which indicated that they were involved in aggressive encounters, most likely intrasexual competition. Although my data indicate that wounds to the head and neck regions of males are inflicted by females and, therefore, indicative of intersexual aggression, the presence of these wounds on males do suggest that the males are at least attempting to mate. Thus, Knudtson's and Thompson's arguments are not necessarily mutually exclusive. It is quite possible that, through aggressive interactions, some males have been excluded from certain haul-out locations but this does not necessarily mean that the males are also being excluded from all mating opportunities.

In this study, an all male haul-out group, including scarred and wounded males, existed at the periphery of the Goulet (outside of the territory areas). The presence of wounds on these males does suggest that they were involved in mating activities. Although these males did not behave as territorial males, they

may have been employing some other "satellite" strategy and probably follow females from the Barachois to mate with them.

Alternate mating strategies have been reported for subordinate males of many species. These behaviours can include subordinates mimicking female behaviour to "sneak" into territories (Gross 1982), smaller, subordinate males intercepting females as they approach territorial males (Howard 1978, 1984), and non-territorial males waiting to intercept females as they leave territories (McVey 1988). The Goulet (all male) harbour seal group at Miquelon, followed the pattern of haul-out found in the Nursery group, in which fewer animals hauled out during the mating period (Weaning Period). There was no increase in fighting nor displaying during the Weaning Period which suggests that these males were not moving into the territory areas. Therefore, it is possible that they may have been intercepting and following females out of the Barachois to mate with them there. None of the males in the all-male haul out group could be sampled for inclusion in the paternity analysis and, therefore, there is no way to compare the reproductive success of males which might be employing a different strategy.

It appears that some male harbour seals are exhibiting a moderate level of polygyny. However this does not mean that females must necessarily only mate with one male per breeding season. It has been observed that the captive female mated with more than one male each breeding season (Chapter 2). Although fathers for a majority of pups could not be identified (Chapter 3), it was possible

to compare their paternal bands. Of the six pups caught in 1986, none had fathers in common (Chapter 3). Not only does this indicate that the level of male polygyny was low but it also suggests that not all females are selecting similar males with whom to mate. There are several possible explanations. Females could mate with males that they encountered randomly, they could choose mates, they could have mated with more than one male in a season, as in some other female phocids (*e.g.* Boness and James 1979, Campagna *et al.* 1993), or they could be doing all of the above.

As female harbour seals in the Nursery area leave the Barachois, they must pass through several territories and encounter the territorial males. Perhaps it is easier to submit to copulating with many of these males rather than risk injury, as hypothesized for northern elephant seals which mate on land (Le Boeuf and Mesnick 1990). However, seals are extremely mobile in water and, therefore, it would seem that harbour seal females could easily evade males there, as has been suggested for aquatically mating puffins (*Fratercula arctica*, Creelman and Storey 1991).

Interestingly, CM had fathered a pup in the 1985 sample and one in the 1986, and his territory was not on a route taken by females to and from the Nursery, indicating that the females may have been exerting some mate choice. Although the purpose of the male harbour seal displays at Miquelon is quite clearly to establish boundaries, the greatest rate of displaying occurred at a time when

there were few animals hauled-out but there would have been movement of animals toward haul-out locations. Therefore, it is possible that females could be assessing males, as they and their pups pass through territories. Unfortunately, it is not possible to determine whether paternity outcomes reflect female mate choice or multiple matings with sperm competition, given that copulations were never witnessed. Presumably, better quality males generally hold better territories, and it is difficult to assess whether females are selecting males on the basis of their displays or the attributes of their territories. Unfortunately, it is not possible to determine on what basis female harbour seals would be selecting males.

I hesitate to label the mating system of harbour seals as polygynous (as defined by Emlen and Oring 1977) because females may mate with more than one male. From the male perspective, it does appear that there is some degree of competition for mates, suggesting that they are behaving polygynously, although no single male or males were highly successful.

Most interesting, is the finding that there appears to be at least one, and possibly two, other mating strategies among male harbour seals at Miquelon. Some males are clearly defending aquatic ter.itories from which other males are excluded. Other males appear to be employing another, satellite strategy (bachelor males in the Goulet) and a potential third strategy may exist. Davis and Renouf (1987) observed terrestrial behaviour of mixed haul-out groups and report

that chasing was never a component of male-male fights, and males did not leave the terrestrial groups to engage in fights. This is different from the male behaviour reported here and suggests that the males they observed were not defending aquatic territories. They do not report the aquatic displays described by Sullivan (1981) and in this study. The purpose of their study was to describe terrestrial spacing patterns and interactions between seals and, therefore, it is possible that they were observing non-displaying males. Another possible interpretation is that male mating strategies vary with the degree of female clumping within haul-out sites and/or topography of the area.

The situation described by Davis and Renouf (1987) was similar to that observed in the South Social observation area in this study. Their haul-out group was situated on the sandflats, in a location where the channel is exceptionally wide, much like the South Social location. Therefore, their study location and the South Social were more similar to Sable Island than the Nursery and Goulet observation sites.

On Sable Island, Boulva and McLaren (1979) reported that "large solitary males are scattered at about 1-km intervals along the beaches" (p. 7) and Godsell (1988) did not find sexually-segregated haul-out groups there. This is more like the situation described by Davis and Renouf (1987) and the South Social. Possibly, the availability of unlimited haul-out space along long stretches of beach, in combination with the lack of discrete aquatic limits, like the channels in and

around the Nursery, has reduced the ability of male harbour seals to establish aquatic territories in a boundary-less medium, as suggested by Stirling (1983) and Bartholomew (1970). In that sort of topography, it may be a better strategy to scramble, or search, for mates.

Miller (1975) found that territorial male New Zealand fur seals responded differently to intruders, depending on the degree to which territory boundaries were delineated topographically. Not only were trespasses more frequent in topographically poorly-defined territories, but territorial males were also more tolerant of these trespasses. Males in territories with boundaries which were well-defined by topographical irregularities were far less tolerant of trespasses. Gentry (1970; as cited in Miller 1975) also found that variations in topography were used to delineate territory boundaries in Steller sea lions and that these boundaries did not change over time. In both species, responses to intruders were most frequent when boundaries were rigidly defined by topography.

At low tide, the shoreline of the Nursery area channels at Miquelon create limits to the aquatic territories. Seals always enter haul-out areas through the water rather than by crossing over the sandflats. Despite the fact that the Barachois becomes one large tidal lake at high tide, the movement of seals through the study sites at high tide was primarily restricted to the deeper waterways which became the channels at low tide. This restricted movement suggests that territories are functionally "hard-edged" (surrounded by habitat

which intruders will not, or cannot, enter) which should reduce intruder pressures (Stamps, Buechner and Krishnan 1987). Therefore, it is possible that the cost of defending aquatic territories in this type of topography is lower than would be expected if males tried to defend "soft-edged territories" in which no clear topographical limits exist. It is tempting to argue that males which have been excluded from territorial areas (areas in which females are encountered at a higher rate) adopt one of two possible alternate strategies. They can either act as satellites and remain at the periphery, which the males in the all-male group in the Goulet may be doing, or they can search for mates, as in scramble competition, which was not apparent in this study but could be happening elsewhere. Thus, it would appear that male harbour seals have a low level of polygyny but that there is some plasticity in the form that the mating system takes.

CONCLUSIONS

Most studies of harbour seal behaviour during the breeding season are based on observations of terrestrial haul-out patterns and interactions between individuals. Several authors have speculated on the mating system of this species based on terrestrial observations (*e.g.* Knudtson 1977, Boulva and McLaren 1979, Slater and Markowitz 1983), but both Thompson (1989) and Godsell (1988) have argued that because most agonistic encounters, as well as mating, occur in the water, little can be concluded by observing male terrestrial behaviour. The results of this study clearly support their argument. Territorial males spent little time hauled out, particularly during the Weaning Period, when mating occurs, and a majority of interactions between the territorial males and other seals occurred in the water.

This is the first study of male harbour seals in which territory boundaries have been mapped. Displays, similar to those used here to map territories, have been described or mentioned by several authors (*e.g.* Sullivan 1981, Almon 1988, Thompson 1988), and all seem to agree that they play a role in the mating system. Sullivan (1981) suggested that these displays were a tool with which females could assess male quality but he did not give them a significant role in interactions between males. The results of this study indicate that displays are threat signals and their primary function is to exclude males from territories.

Nonetheless, females could also be using the displays as a measure of male status and quality to assist in mate choice.

Based on the behaviour of adult male harbour seals at Miquelon, males appear to be adopting at least two mating strategies, and possibly a third. Some males are defending aquatic territories through aggression and threat displays at boundaries, while others could be using an alternate, perhaps "satellite" strategy. Females move throughout the Barachois during the breeding season and, therefore, have ample opportunity to assess male quality, either through displays or through the outcomes of intermale aggression. Although the data are sparse, the results of paternity tests suggest that the level of male polygyny is low.

Because this species mates in the water, and females may copulate with more than one male, it is difficult to assess the impact of female behaviour on the mating system. To address this question, it would be necessary to determine with which males females mate and which males sire pups. Obviously this is no small feat, in a species that mates underwater. Bartsh *et al.* (1992) devised an ingenious method to determine which male Weddell seals were copulating. By applying coloured grease to the fur around the penile opening, they could determine which males mated with which females by observing coloured grease transferred to females.

It appears that males at Miquelon are behaving differently from males in other regions as territoriality has not been described in this species before. The

difference between male behaviour at Miquelon and other locations may be caused by variations in the environment/topographical features of haul-out environments. Territorial behaviour at Miquelon is likely facilitated by narrow channels through which females must pass. The channels create hard-edged boundaries which should reduce intruder pressure and, therefore, territory defence should be less costly than if territories were soft-edged, as would be expected along a homogeneous haul-out site. In order to test this suggestion, it would be necessary to compare behaviour of identified harbour seal males in varying topographical habitats.

To better understand the mating strategies of male and female harbour seals, future research would have to include behavioural observations and radio-tracking of territorial males and those males adopting an alternate strategy. In addition to using paternity testing to assess male reproductive success, it would also be useful to tag females, firstly, to determine the extent of their movement during the breeding season and, secondly, to determine if tagged males move with them.

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APPENDIX I: Solutions used during DNA fingerprinting procedure.

7 X Digest Solution
- 6% SDS
- 500mM NaCl
- 200mM Tris, pH 8.0
- 50mM EDTA

TE Buffer
- 10mM Tris HCl, pH 8.0
- 1mM EDTA, pH 8.0
- (pH adjusted to 8.0 with NaOH and HCl)

Denaturing Solution
- 0.5M NaOH
- 1.5M NaCl

Pre-Hybridization Solution
- 45ml distilled water
- 2.5ml 10% SDS
- 2.5ml 20 X SSC
- 2g PEG 6000 (Polyethylene glycol)
- 50ul 50mg/ml heparin in TE
- 50ul tRNA (approx. 1mg/ml in TE)

Wash Solution
- 1 X SSC
- 0.1% SDS

Neutralizing Solution
- 0.2M Tris-HCl, pH 7.5
- 0.1 X SSC
- 0.1% SDS

APPENDIX II: Tag placement on pups (P), juveniles (J), adult females (AF) and adult males (AM) and percent resightings in the year tags were placed (Tag Year) and over all years subsequent years.

YEAR	AGE CLASS	# OF TAGS	NUMBER RESIGHTED	
			TAG YEAR	SUBSEQUENT YEARS
1984	P	14	8	3
1985	P	34	8	7
	J	2	1	2
	AF	3	1	1
	AM	1	1	1
1986	P	29	10	8
	J	1	0	1
	AF	11	2	6
1987	AM	1	1	1
1988	P	6	0	1
	J	2	0	0
	AF	2	0	1
	AM	1	1	1